Vol. XLI No. 1

THE BOTANICAL GAZETTE

January, 1906

Editors: JOHN M. COULTER and CHARLES R. BARNES

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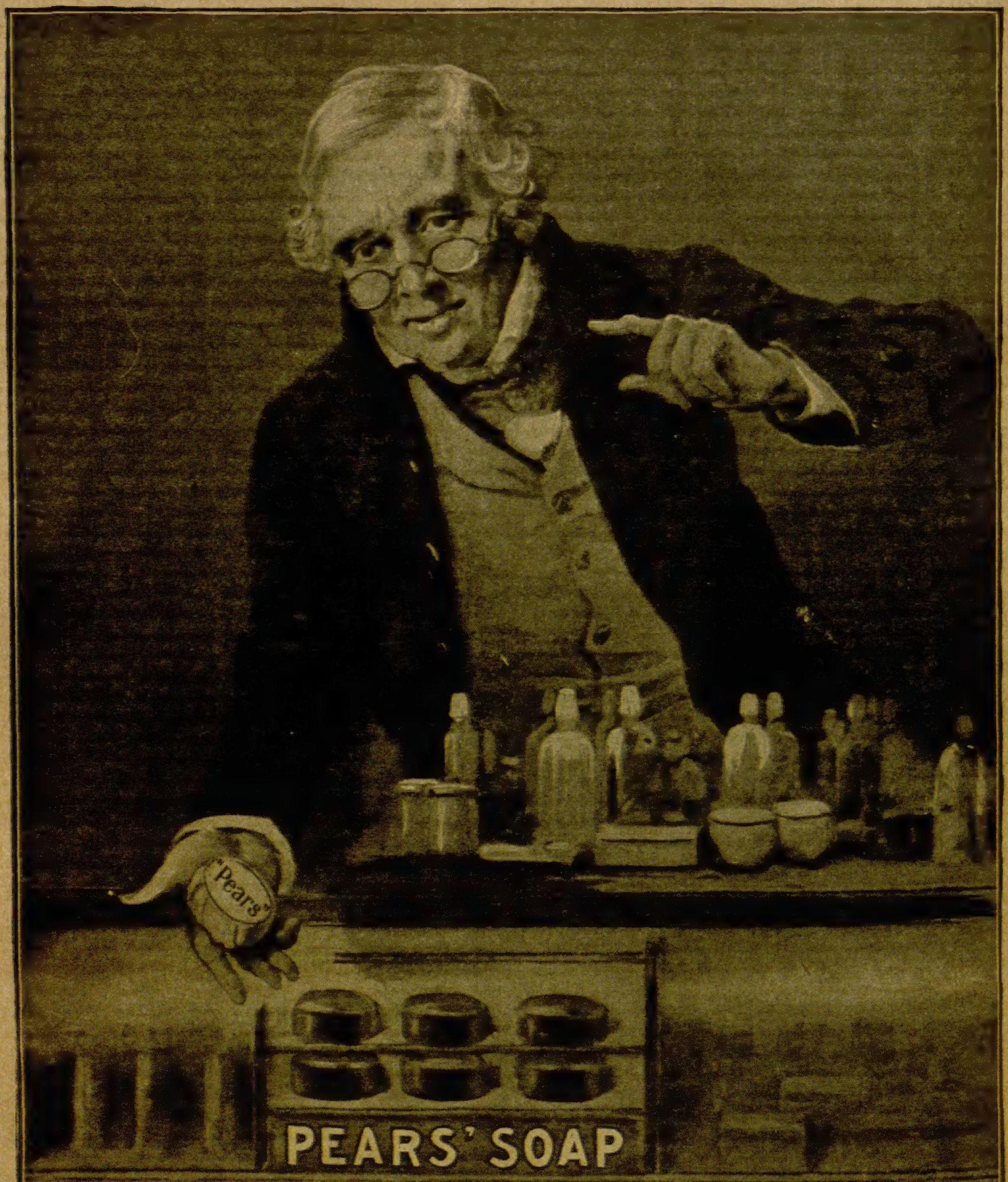

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VOLUME XLI

JANUARY—JUNE, 1906

WITH SIXTEEN PLATES AND SIXTY-TWO FIGURES

CHICAGO, ILLINOIS

PUBLISHED BY THE UNIVERSITY OF CHICAGO

1906

Mo. Bot Garden
1906

PRINTED AT
The University of Chicago Press
CHICAGO

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DATES OF PUBLICATION.

No. 1, January 26; No. 2, March 3; No. 3, March 31; No. 4, April 28; No. 5, May 31; No. 6, June 30.

ERRATA.

P. 10, line 19, for (10) read (11).
P. 14, line 2 from bottom, for nternodes read internodes.
P. 15, line 4 from bottom, for Contribution read Contributions.
P. 75, line 16 from bottom, for East read West.
P. 76, line 4 from bottom, for Perti read Petri.
P. 167, line 19, for stand read stain.
P. 168, line 13 from bottom, for comma read in.
P. 177, line 1, after was insert in.
P. 178, line 12 from bottom, for its read their.
P. 179, line 11 from bottom, after paraphyses insert cryptostomata.
P. 180, line 3, for The read Two.
P. 340, line 12, for heller read Heller.
P. 353, line 3 from bottom, for Petrostemonaceae read Podostemonaceae.
P. 370, line 13, for Alluim read Allium.

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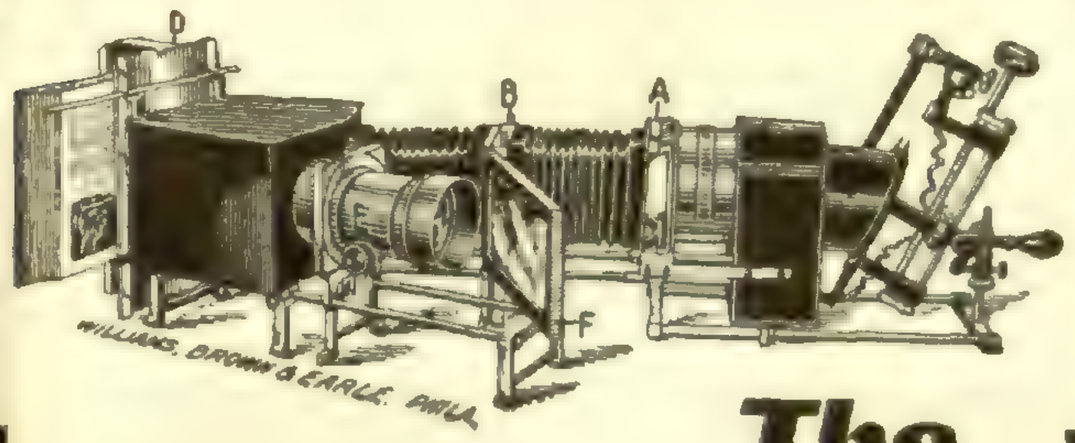

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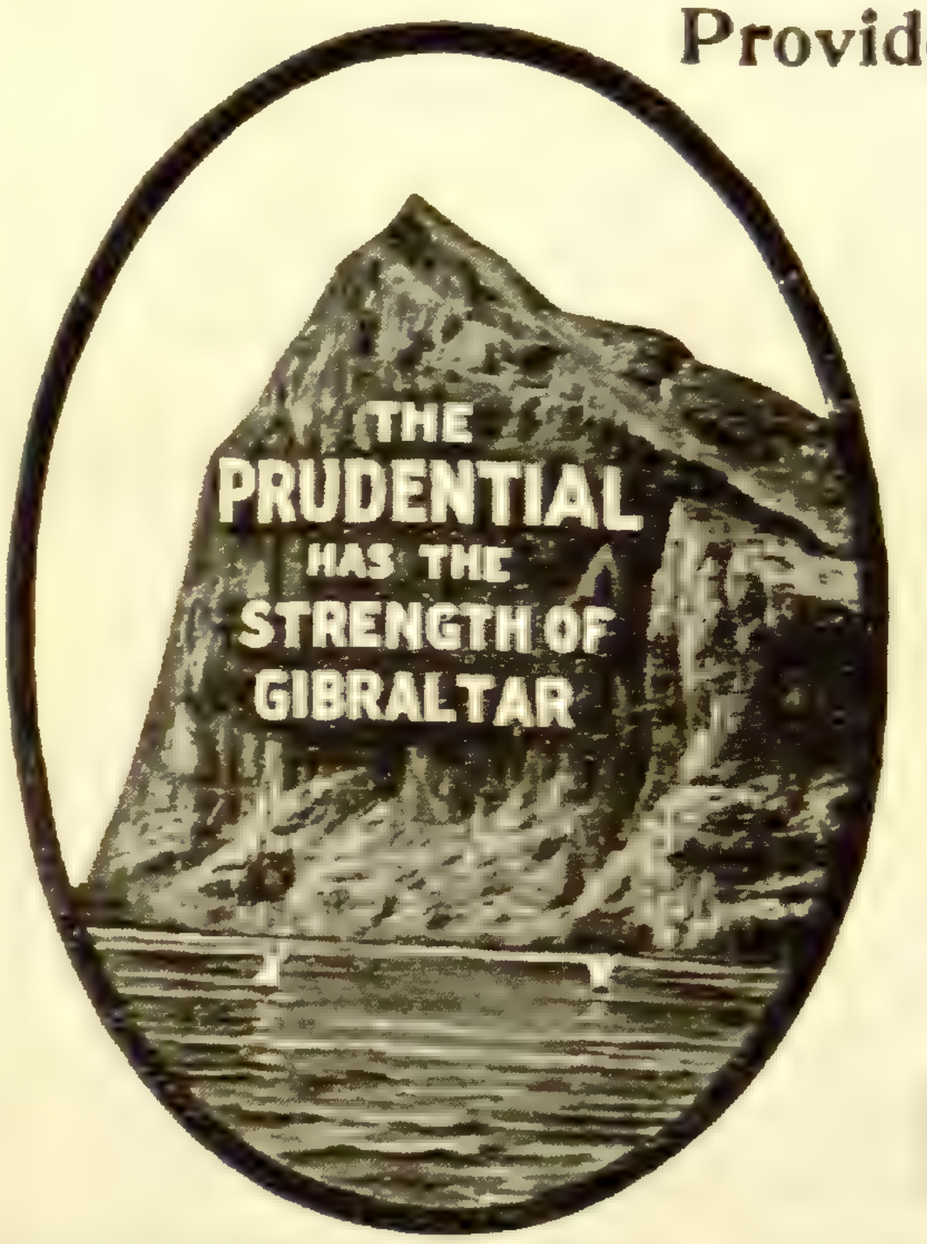

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VOLUME XLI NUMBER 1

BOTANICAL GAZETTE

JANUARY, 1906

THE NODES OF GRASSES.[1]

MINTIN ASBURY CHRYSLER.

(WITH PLATES I AND II)

ALTHOUGH the stems and leaves of grasses have received a good share of attention from anatomists, and the bundles of the internodes are perhaps sufficiently well known, the nodes have been largely neglected. The reason for this may be the supposed difficulty of unraveling the tangle of bundles found in the node, or the obstacles which the sclerified tissues offer to the preparation of satisfactory sections. Yet the nodes are probably the most interesting regions of the grass stem, for they lack the comparative uniformity of the internodes. Since the application of the celloidin method to hard tissues the difficulties of cutting the necessary serial sections have been removed, so that we are now in a position to know intimately the structure of these critical regions of the stem.

The object of the present account is to trace the course of the bundles of the grass stem, and to discuss the significance of certain structures which make their appearance at the nodes, in particular the amphivasal bundles and cambium. The investigation has been confined to forty-five genera, but since these represent the eleven largest tribes and there is a considerable degree of uniformity in structure, the account is believed to represent the family fairly from the standpoint chosen.

The salient features may best be brought out by the description of types selected to illustrate certain points. The first to be considered represents a medium condition as regards both taxonomic position and ecological relations.

[1] Contributions from the Phanerogamic Laboratories of Harvard University. No. 3.

Avena barbata.—At a short distance above a node the stem presents a simple structure compared with that of many grasses, showing just two circles of collateral bundles surrounding a central cavity. The inner circle of bundles lies at the periphery of the central cylinder, which in this genus is not clearly marked off from the cortex. The bundles of the outer circle lie between groups of cells, which above the leaf-sheath bear chlorophyll; they are considerably smaller than the bundles of the inner ring, and from comparison with corresponding bundles in other genera must be regarded as cortical bundles. In the upper part of a node these assume an amphivasal structure and immediately anastomose with one another and with bundles of the inner circle. But just at this level the structure of the stem is further complicated by the entrance of bundles of the leaf-trace, the course of which must now be described. The leaf-sheath in this species extends a little more than 360° around the stem, and contains, as do most of the genera examined, bundles of two distinct sizes: larger ones, alternating with others which are less than half as great in diameter. These two kinds of leaf-trace bundles have a different course in the stem. The larger bundles undergo a profound modification as they enter the stem, as may be seen by comparing *figs.* 1 and 2, in which the magnification is the same. As such a bundle enters the stem it rapidly increases in size, owing to increase in the number of xylem elements. Most of the added elements are tracheids with more or less suberized scalariform or reticulate thickenings, but some parenchyma cells are also present. These elements gradually extend around the sides of the phloem until this is surrounded by xylem, except a small area on its outer side, which is generally occupied by sclerotic fibres belonging to the group which is so well marked in the sheath (*fig.* 1). In certain species, e. g., *Lolium perenne*, these fibres disappear, and the phloem is completely surrounded by xylem. These bundles evidently must be placed in the amphivasal class. As *fig.* 2 shows, the phloem enlarges very little, but the xylem increases so much that the bundle may be five or six times as broad in the middle of the node as it is in the leaf-sheath. These bundles are by far the most conspicucus objects in a cross-section of the stem at this level. They have a spindle shape which is not due to their oblique course, for they slant

very slightly from the vertical and do not dip deeply into the central cylinder. The xylem consists so largely of tracheids running irregularly and mixed with parenchyma, that the mass has a considerable resemblance to transfusion tissue. Apparently, this region in a bundle forms an important water-storing organ. A further peculiarity of the bundle at this level is the presence of a distinct bundle-sheath or endodermis,[2] consisting of cells whose walls are reticulately thickened and suberized. As these larger leaf-trace bundles descend through the node, branches from the anastomoses mentioned above extend outward between the leaf-trace bundles, and probably anastomose with these, though the fusion is not so plain as in *A. sativa*. Below this level the bundles gradually resume the ordinary collateral shape, lose their endodermis, and run down in a single circle through the internode as already described. The smaller leaf-trace bundles also undergo some expansion as they penetrate to the boundary of the central cylinder, but throughout their course they may be distinguished from the larger bundles, not only by their size but by their early turning outward into the cortex and running down to the next node as the cortical bundles described above.

Though it is not plain in *A. barbata* that the larger leaf-trace bundles are joined, soon after their entrance into the stem, by other bundles of the node, in *A. sativa* and in many other grasses it may clearly be made out that on each flank of the leaf-trace bundle another strand applies itself, swinging through an angle, so that its phloem first joins on, then its xylem. In some species, e. g., *Arundo Donax*, two or more bundles join on each flank of the leaf-trace bundle.

Certain features of the cortical strands are more clearly seen in *Panicularia americana*. The cortical nature of these strands is unquestioned, for they run quite outside the central cylinder, in a wide area of lacunar parenchyma (*fig. 7*). As they reach the upper part of a node they anastomose with one another so as to form a transverse ring or girdle (*fig. 8*), which, at a slightly lower level, sends branches to the bundles of the central cylinder, forming nearly or quite amphivasal bundles, though some of them very soon resume

[2] The term endodermis is here used in the general sense employed by VAN TIEGHEM, rather than in the histological sense proposed by other writers.

the collateral structure which is characteristic of all the bundles of an internode. Further down in the node, the bundles of the leaf-trace enter the central cylinder and the smaller of these anastomose with branches of the nodal complex, and then turn outward to run down through the internode as cortical bundles. The larger leaf-trace bundles behave as in *A. sativa*.

In *Leersia oryzoides* it may plainly be seen from a series of sections, that as each of the smaller leaf-trace bundles enters a node, it is joined by two small bundles from the nodal complex, and this reinforced bundle proceeds downward through the sclerified cortex.

Whether the smaller leaf-trace bundles run down through the cortex, or in the outer region of the central cylinder of an internode, cannot in all cases be determined with certainty, for the boundary of the central cylinder is often poorly marked, and the cortex may be a very narrow zone. Or the boundary of the central cylinder may be marked by a narrow sclerenchymatous ring, and the bundles may lie along this, projecting either towards the inside or the outside. But the position of these bundles inside or outside the central cylinder appears to be a matter of indifference, and in either case they pursue a different course from that of the larger bundles. In a general way it may be stated that the smaller bundles of the leaf-trace, after fusing with bundles from the nodal complex, run downward through the next succeeding internode in the cortex or at its inner border, and at the next node below join with the bundles of the central cylinder. Species to which this statement applies are: *Zizania aquatica*, *Leersia oryzoides*, *Avena barbata*, *A. sativa*, *Panicularia americana*, *P. nervata*, *Agropyron caninum*, *Elymus americanus*, *Triticum sativum*.

The course of the leaf-trace bundles in the grasses, as here described, differs in several respects from the course of such bundles in other families, even in so closely related a family as the sedges, recently described by PLOWMAN (**11**). VAN TIEGHEM'S second class of cortical bundles (**6**, p. 751) corresponds the most closely, and is thus described: "Le faisceau médian de la feuille, qui en prend trois, entre directement dans le cylindre central, tandis que les deux latéraux descendent dans l'écorce pour n'entrer dans le cylindre

qu'au nœud suivant." See further his remarks on the monocotyledons.

Phalaris arundinacea, like Avena, has the bundles of its internode crowded into an annular area surrounding the fistular pith. As these reach the node they anastomose extensively, and at the same time assume the amphivasal condition, which is shown with especial clearness in the varicty *variegata*, *figs. 3* and *4*, the latter more highly magnified in order to show the tendency for bundles to form nests of three or more, enclosed by an armor of sclerified fibres. These amphivasal bundles, though abundant in the nodes, are absent from the internodes.

Arundo Donax may be mentioned as typical of species having several circles of bundles surrounding a central cavity. As the leaf-trace bundles enter the stem they swell out, though not to so great an extent as in Avena. The xylem completely encloses the phloem, and the usual suberized sheath of cells with reticulately thickened walls becomes visible. Farther down in the node the leaf-trace bundle is joined on each flank by one or more cauline bundles. The bundles of the latter class are provided with a sheath of heavily thickened cells, and some of them appear to pass through the node without anastomosing with other bundles, though this condition is rare in the members of the family which have fewer bundles. In accordance with the greater thickness of the solid part of the stem, the leaf-trace bundles penetrate more deeply into the central cylinder than in such genera as Avena, making their general course conform more nearly to the palm type of VON MOHL. The number of circles of bundles in an internode appears to be dependent on the size of stem characteristic of the species, and to have little value in establishing relationships.

Grasses with a solid stem conform even more nearly to VON MOHL'S type, for the largest leaf-trace bundles penetrate nearly to the center of the stem, before curving outward and downward toward the periphery of the central cylinder. STRASBURGER (4) has given an excellent account of the course of the bundles in *Zea Mays*. He distinguishes leaf-trace bundles of five different ranks, and finds that the largest of these penetrate most deeply into the stem, while the smallest merely reach the periphery of the central cylinder.

The increase in complexity of the leaf-trace system over the condition found in Avena, seems to be associated with the greater size of the leaf-sheath in Zea; just as a large stem generally has several circles of bundles, so a heavy leaf has a better developed bundle system. Most of the bundles in an aerial node of Zea are collateral, leaving out of consideration the swollen leaf-trace bundles. STRASBURGER (**4**, p. 348) finds amphivasal bundles at the point of origin of axillary buds and adventitious roots; I have confirmed his observation in the former case. Much larger and more numerous amphivasal strands are however to be found in the nodes of the axillary branches bearing the ear of corn. One of these bundles is represented in *fig. 5*. It is only in the leafy part of the branch that these occur, for in the "cob" the bundles are collateral, with an exceedingly well-developed phloem, doubtless associated with the transfer of elaborated food. The amphivasal bundles of these branches are as usual bundle fusions, and their occurrence in the reproductive axis of a plant showing few elsewhere, seems to be a point of some significance, especially when we consider that Zea is probably a highly organized member of the family.

Zizania aquatica merits special attention on account of certain features which may be regarded as primitive, e. g., the six stamens. In an aerial internode a narrow cortex surrounds the hollow central cylinder, and the two are separated by a ring of sclerified cells. Partly imbedded in this ring are a number of small bundles, some of which project into the cortex and on account of their origin must be regarded as cortical bundles. All the bundles are collateral, and those inside the sclerotic ring lie at different depths within the central cylinder. As would be expected from the aquatic habitat of the plant, the xylem is reduced; in fact, in some instances, it is represented only by a cavity, and in all cases it has its vessels very slightly lignified. The phloem does not share in this reduction. As the node is approached the bundles at the periphery of the central cylinder anastomose, at the same time becoming amphivasal, and a number of transverse strands join up some of the inner bundles of the stele with one another. At this level the leaf-trace bundles enter the stem; they are of at least three ranks, and of these the largest penetrate into the central cylinder, enlarging on the way,

owing to increase in the xylem elements, which come to enclose the phloem more or less completely, as already described for Avena. A little further down they are joined on the flanks by several bundles from the internode above, each leaf-trace bundle with its tangle of contributing bundles forming a complex bunch of vascular tissue. A suberized endodermis surrounds the leaf-trace bundles in the node. The second rank of bundles of the leaf-trace also enter the central cylinder, where they are joined by other bundles, but soon return to the periphery of the central cylinder, where they give rise to some of the bundles which run through the next internode below on the border line between the sclerotic ring and the cortex. The smallest bundles of the leaf trace go no deeper than the sclerotic ring, and here fuse with other bundles found in this zone. Thus, the course of the bundles of different rank agrees with what STRASBURGER found in Zea. In the lowest part of the node, the very numerous bundles of the sclerotic ring anastomose and proceed downward, greatly reduced in numbers, while the bundles inside the central cylinder also become much fewer, owing to completion of the fusion of the large leaf-trace bundles with cauline strands.

The basal region of the stem has a cortex which differs from the aerial parts in being much broader and more spongy, on account of the large intercellular spaces. At any of the basal nodes the central cylinder is bounded by an endodermis, consisting of a single layer of rounded cells with suberized walls (*fig. 6*). Inside this is a narrow zone of vascular tissue, whose elements run circularly; then a wide zone, consisting of small bundles running vertically, and so closely packed together that it is generally impossible to distinguish their limits. Bounding the two vascular rings on the inside is a band of sclerified cells which are in contact with the pith. As the photograph shows, leaf-trace bundles make their way into the central cylinder through wide gaps in this four-layered ring, and it may be clearly seen, even in unstained sections, that along the edges of such a gap the external suberized endodermis is continuous with the inner sclerified layer. All of the large leaf-trace bundles pass through such gaps, but the roots leave the central cylinder without causing a gap, as has been observed in plants of various groups. In the pith of the central cylinder are scattered many

bundles, nearly all of which are amphivasal, and where the nodes are crowded, as they are at the very base of the stem, the amphivasal condition is retained by bundles from one node to another, though in the more elongated internodes, found a little higher in the stem, only collateral bundles occur. The contrast between the upper and the basal nodes is indeed striking, for the former show no amphivasal bundles running longitudinally in the pith, except the enlarged leaf-traces. This feature of the aerial node may be partly accounted for by the thinness of the diaphragm in which run the anastomosing bundles, which are generally amphivasal.

AMPHIVASAL BUNDLES

Although these have been repeatedly reported as occurring in the subterranean stems of monocotyledons (see STRASBURGER, **4**, p. 348, footnote; DEBARY, **2**; SCHULZE, **5**)[3], the only references to their occurrence in grasses that have been found are by STRASBURGER (**4**) and JEFFREY (**9**). DUVAL-JOUVE (**1**) figures the rhizomes of many grasses, but shows no amphivasal bundles. Yet an examination of the nodes of some of the same species shows that the bundles in question occur here. Two sorts must be distinguished in this family: (1) the swollen portion of a leaf-trace bundle, the xylem consisting largely of a mass of tracheids running irregularly; (2) the type usually figured, the xylem forming simply a ring of vessels. The features of the first class have been described under Avena. Such a bundle is always enclosed by an endodermis which generally has pitted or reticulated walls, and shows, in addition to the ordinary metaxylem elements, a large number of reticulated tracheids, which almost or quite enclose the phloem. All the species examined show these bundles, from hydrophytes such as Zizania to xerophytes such as Ammophila, and there appears to be no relation between the size which a bundle attains at its widest part and the condition under which the species grows. If, as already suggested, these bundles serve to store up water, it might be expected that they would be poorly developed in aquatics, but the only peculiarity of the bundles found in such plants is the slight lignification of the xylem, a char-

[3] Since the above was written, HOLM has reported the occurrence of amphivasal bundles in the rhizome and also the aerial stem of *Croomia pauciflora* (Amer. Jour. Sci. **20**:50–54. 1905).

acter shared by all the bundles of such plants. Quite commonly, the bundles are surrounded by a layer of parenchyma rich in chlorophyll. This suggests that the node is an active assimilating organ. Bundles of the second class—amphivasal bundles as usually understood—are found in the nodes of the great majority of the grasses examined, but are especially numerous in the following species: *Coix lachryma, Paspalum stoloniferum, Panicum sanguinale, Sorghum halepense, Leersia oryzoides, Phalaris arundinacea, Calamagrostis canadensis, Avena barbata, Panicularia americana, Panicularia nervata, Festuca arundinacea, Triticum sativum.* The fact that they are practically confined to the nodal regions, suggests that they are associated with bundle fusions, and this assumption has been amply borne out by observation. Further, since the bundles which fuse are usually traces of leaves which come off higher up, it appears that the occurrence of such bundles is to be referred to the leaves. The closed mode of venation, prevalent in the monocotyledons, involves that a large number of bundles shall run down parallel to one another through the petiole or sheathing base of the leaf. In the grasses the numerous bundles are accommodated in the leaf-sheath, which frequently encircles the stem for somewhat more than 360°. The large number of bundles cannot at once find room in the vascular ring, which we may believe constitutes the primitive stele in both dicotyledons and monocotyledons, according to the results of JEFFREY (**8**), and CHRYSLER (**10**). Hence the leaf-trace bundles, or some of them, pass into the inside of the central cylinder, and sooner or later join other bundles. It will be readily seen that a bundle lying in the pith has a better chance to orient itself with regard to some other bundle which it may join, than has a bundle which merely fits itself into a gap in a vascular ring (e. g., the leaf-trace of a fern such as Adiantum). Hence it is not surprising to find that before two bundles of a monocotyledon fuse, they swing around, so that phloem fuses with phloem, and the xylem accordingly surrounds the compound bundle. How far beyond the point of fusion of the bundles this amphivasal condition persists, is a feature which varies greatly. In most of the grasses the collateral structure is soon resumed, but the example of Zizania shows that, at the base of the stem where the nodes are crowded, the amphivasal condition

may continue through several internodes. This probably accounts for the prevalence of these bundles in the rhizomes of monocotyledons, where they were first observed. A comparison of the aerial and subterranean nodes of the grasses under study has not yielded results of great significance; most species show no noticeable difference in the number of amphivasal strands in the two cases. But in *Andropogon furcatus*, *A. scoparius*, *Chrysopogon avenaceus*, *Zizania aquatica*, and *Phleum pratense*, the amphivasal strands are distinctly more numerous in the basal nodes. No examples of the opposite condition have been found. QUEVA found in Gloriosa (**7**) that the amphivasal bundles are connected with the origin of a branch. Among the grasses, *Phalaris arundinacea*, *Paspalum stoloniferum*, *Sporobolus Wrightii*, *Coix lachryma*, and *Zea Mays* show these bundles at the point of origin of branches, but in other species only collateral bundles could be discovered at these places. Too much importance should not be attached to the few cases named, in view of the fact that the sedges uniformly show amphivasal bundles associated with leaves and not with branches, as PLOWMAN has shown (**10**). This is one reason for considering the grasses a more specialized group than the sedges; in fact it may be premised that the amphivasal condition originally connected with leaf-traces has in the Gramineae spread to the branches. The occurrence of amphivasal bundles in the leafy reproductive axis of Zea, while they are rare in the main stem, deserves emphasis. While many of the grasses show amphivasal bundles in all the nodes, in this highly developed genus the bundles in question have nearly disappeared from the ordinary nodes, but have persisted in the conservative region named. STRASBURGER proposes (**4**, p. 348) a physiological explanation for the occurrence of these bundles, viz., that the amphivasal structure is favorable for the taking up of reserve materials stored in a rhizome, but this explanation is not in accord with the accepted view that elaborated food is carried not by the xylem, but by the phloem. Examination of serial sections leads to the opinion that the mechanical necessities of bundle fusion rather than considerations of absorption of food have been the determining factor in producing these bundles.

If we accept the view advanced above, that these bundles are to

be associated with the large number of leaf-trace bundles of the monocotyledons, their phylogenetic significance is considerable. The ferns and dicotyledons have a comparatively small number of leaf-trace bundles; amphivasal bundles are absent in the former and rare in the latter group, but are widely distributed in the monocotyledons, which accordingly appear to represent a more recent and highly specialized group. In the most highly organized members of the Gramineae, such as Zea, is shown a tendency to reduce the number of amphivasal strands, but even in such cases they may linger on in the reproductive axis. JEFFREY (9) has called attention to the fact that in highly organized families, such as Iridaceae and Orchidaceae, these bundles disappear even from the reproductive axis.

CAMBIUM.

A feature of the vascular bundles of *Avena barbata*, not so far mentioned, is represented, in *fig. 9*, which shows a bundle from the stem at a distance of about 1^{mm} above one of the upper nodes. That the tissues are immature is shown by the presence of protoplasm and a nucleus in certain of the vessels. The shrunken protophloem is represented by the dark band at the outer edge of the bundle, and the metaphloem as usual has its elements arranged irregularly. Between these elements and the vessels are a number of rows of flattened cells, radially arranged, corresponding well to the cambium of dicotyledons. This feature is not confined to the young stem, as is seen in *fig. 10*, which represents a bundle from above one of the lower nodes of the same plant. A few tangential divisions are to be seen in the leaf-trace bundle shown in *fig. 1*, though cambial activity here is slight. Toward the node and farther up in the internode and sheath, this peculiarity is not shown by the bundles, but at the regions mentioned most of the bundles have a more or less clear indication of cambium.

In the leaf-sheath of *Andropogon argenteum* (*fig. 11*), at a distance of $1–2^{mm}$ above its insertion on the stem the larger bundles show an unmistakable cambial layer. From the small amount of phloem or xylem showing radial arrangement of its elements, it appears that the cambium is functional for only a short time. Sections through the leaf-sheath, cut 5^{mm} above the one shown in the figure, still exhibit a layer of radially arranged cells exterior to the xylem,

but the cells have thicker walls and a rounded outline, indicating that in that region the period of activity of the cells is past. The stem of this plant does not show good examples of cambium, nor do *A. scoparius* or *A. furcatus* show the feature, even in the leaf-sheath.

A further example is shown in *fig. 12*, which represents a bundle from the stem of *Erianthus Ravennae* 1–2^{mm} above the level of insertion of the leaf-sheath. A similar appearance is presented by the bundles of the sheath. The leaf-trace bundles of Zizania frequently show a large amount of their phloem radially arranged, in spite of the fact that dicotyledonous aquatics generally show a marked reduction of their cambium. The examples so far cited include only the more striking instances of cambium found in the family. More or less plain evidences of cambium have also been observed in the following species: (1) In both stem and leaf-sheath; *Coix lachryma, Panicum crus-galli, Avena sterilis, Lolium italicum, L. perenne.* (2) In stem; *Tripsacum dactyloides, Miscanthus sinensis, Pennisetum longistylum, Panicum sanguinale, Leersia oryzoides, Sporobolus Wrightii, Calamagrostis canadensis, Arundo Donax, Avena sativa, Briza maxima, Panicularia americana, Bromus inermis, Triticum sativum.* The occurrence of a cambium in the region just above the nodes in grasses recalls the well-known power which members of this family possess of bending upward at these regions if the stem is by any means laid horizontally. In this connection it is of interest to note that PLOWMAN (**11**) has found only traces of cambium in the sedges, and in line with this, the stems of sedges are unable to right themselves if bent over into a horizontal position. Miss ANDERSSON (**3**) has reported the occurrence of a more or less plain cambial zone in the young plants of representatives of many monocotyledonous families. She calls attention to the similarity between the bundles in the seedling of Lilium and those in Ranunculus. The only grass referred to is Zea, in which the mature bundles often show a radial arrangement of the cells between xylem and phloem, as is illustrated by the well-known figure in SACHS' *Text-book.* From the occurrence of cambium in the tuberous stems of Gloriosa, QUEVA (**7**) has already concluded that the monocotyledons are derived from dicotyledons. In the case of the grasses it would seem that the cambium possessed by the ancestors

has been retained in the regions where it is of use. On the other hand, it may be argued that we have here the first appearance of a feature which in the dicotyledons becomes prevalent. But why should the cambium appear only at the nodes, where it is of use? It may be regarded as almost axiomatic that the need for a structure is not a sufficient cause for its appearance. So it seems more reasonable to read the evidence in the way first suggested, viz., that we have here a relic of a structure which was present in the ancestors of the grasses, but has disappeared from most parts of the plant and from most families of monocotyledons, and is retained above the nodes of grasses in connection with their power of bending at these regions. Thus the evidence favors the derivation of the grasses from ancestors having a cambium.

The stele of the grass stem has evidently departed widely from the primitive protostele or siphonostele. It has been repeatedly shown that reproductive axes are able to retain ancestral characters. An examination of this region in seventeen species of grasses belonging to seven of the tribes has failed to disclose any instances where the stele presents the primitive type described by PLOWMAN (11). This result seems to confirm the opinion derived from other considerations, that the Gramineae represent a more specialized group than the Cyperaceae. These considerations may now be stated categorically:

(1) The grass family has adapted itself to every habitat, from salt marsh to pampas, and shows every gradation in habit from the bamboo downward. The sedges are prevailingly hydrophytes, and few of them attain a considerable size.

(2) The hollow stem characteristic of most grasses has probably been derived from a solid stem such as is present in the sedges and most monocotyledonous families.

(3) Amphivasal bundles are not found in so large a proportion of species nor are they as numerous in an individual in the grasses as in the sedges.

(4) In practically all of the grasses the leaf-trace bundles are of at least two ranks, while the sedges show no such distinction.

(5) The floral axis of the grasses does not present the simple type of stele shown by some sedges.

On the other hand, the open sheath of the grass leaf may be considered to be more primitive than the closed sheath of the sedges. Further, the cambium found in the grasses is here considered to be a primitive feature. But on the whole the Gramineae are to be regarded as a more highly specialized family than the Cyperaceae, though the families are evidently very closely related.

Certain anatomical features of the grasses, such as the distribution of the amphivasal bundles, seem to have an important bearing on the phylogenetic position of the family among monocotyledons, but since the anatomy of the group is as yet very imperfectly known, a discussion of this point would be premature.

SUMMARY AND CONCLUSIONS.

1. The grasses depart considerably from the scheme proposed by VON MOHL for the course of the bundles, chiefly owing to the stem being hollow in most cases. The leaf-trace bundles are of at least two ranks; of these the largest penetrate most deeply as they enter the central cylinder, generally receiving one or more bundles on each flank as they pass downward to the lower part of the node; the smaller leaf-trace bundles do not penetrate deeply into the central cylinder, but after anastomosing with other bundles pass downward either in the cortex or at the inner border of this. At the next node lower, these cortical bundles anastomose with one another, and then with the bundles of the central cylinder which have come from fusions with leaf-trace bundles at the next node above.

2. The leaf-trace bundles, especially the larger ones, undergo a marked change as they enter the stem. This consists in the appearance of a distinct endodermis, and in an increase in the xylem, leading to the formation of a greatly swollen amphivasal bundle. Below the node these bundles resume the collateral type.

3. Amphivasal bundles of the ordinary type, though absent in the aerial internodes, are very commonly found in the nodes, and arise by fusion of collateral bundles which are generally leaf-trace bundles. In some species they are more numerous in the nodes at the base of the plant, and where such nodes are crowded, the bundles may retain the amphivasal condition through successive internodes. The presence of amphivasal bundles in reproductive branches of plants in which these bundles are scarce in ordinary

nodes, points to their being an ancestral feature, which, in highly organized members, has disappeared from most parts of the plant, but is retained in the conservative flowering axis. It appears that the amphivasal bundles so characteristic of monocotyledons, in all probability made their appearance in connection with the entry of numerous leaf-trace bundles into the nodes, but that secondarily, in certain instances, they are found to be related to branching.

4. A well-marked, though generally short-lived, cambium occurs in the bundles just above the node or near the base of the leaf-sheath in certain grasses. This fact is considered to lend support to the view that monocotyledons have been derived from some group possessing a cambium, probably the dicotyledons.

5. The anatomical features of the grasses point to their being a more highly specialized family than the sedges.

This investigation has been carried on in the Phanerogamic Laboratories of Harvard University. I am indebted to Professor G. L. Goodale for material, and to Professor E. C. Jeffrey for material and for advice during the progress of the work.

Harvard University.

LITERATURE CITED.

Note.—Practically nothing bearing immediately on the subject of this research has been found in the older literature, so it is not cited here. References to it may be found in DeBary (2), and the text of Kny's *Wandtafeln*.

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EXPLANATION OF PLATES I AND II.

PLATE I.

FIG. 1. *Avena barbata;* leaf-trace bundle in the leaf-sheath 1^{mm} above its insertion on the stem. ×130.

FIG. 2. Same; swollen and amphivasal condition of a leaf-trace bundle in the middle region of node. ×130.

FIG. 3. *Phalaris arundinacea;* section through upper part of a node; near the outside are the leaf-trace bundles, alternating large and small; just internal to these are the nests of amphivasal bundles. ×30.

FIG. 4. Same; one of the nests of amphivasal bundles more magnified; on three sides are leaf-trace bundles. ×150.

FIG. 5. *Zea Mays;* one of the large amphivasal bundles from a node of a reproductive branch. ×115.

FIG. 6. *Zizania aquatica;* part of stele and cortex in basal region of the stem. Two gaps with their leaf-trace bundles are visible. ×35.

PLATE II.

FIG. 7. *Panicularia americana;* section 1^{mm} above node, showing two cortical bundles. ×45.

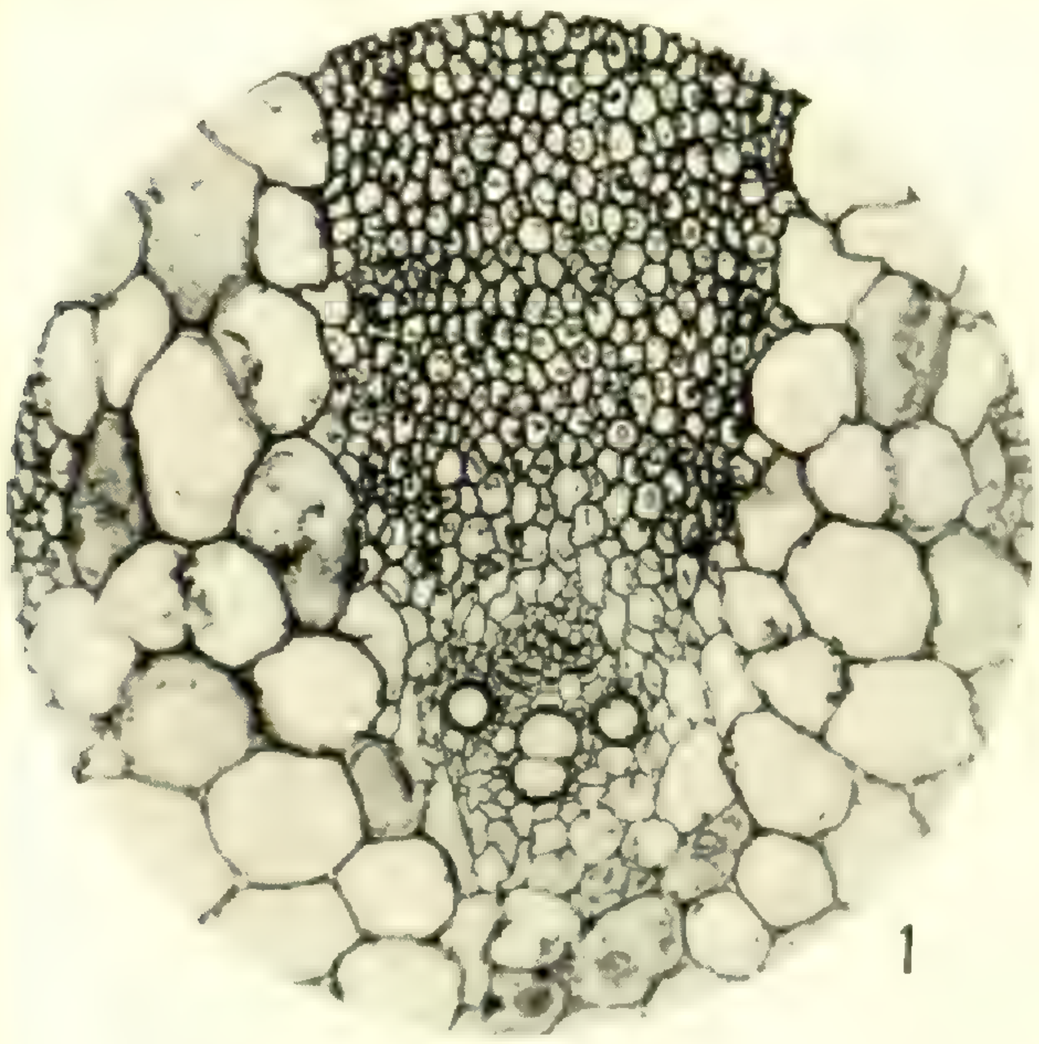
FIG. 8. Same; section from upper part of the node, just above insertion of leaf-sheath. The cortical bundles are connected by a ring-shaped anastomosis. ×45.

FIG. 9. *Avena barbata;* bundle from a young stem 1^{mm} above insertion of the leaf-sheath, showing cambium. ×150.

FIG. 10. Same; bundle from the same position in a mature stem. ×150.

FIG. 11. *Andropogon argenteum;* bundle from the leaf-sheath 1–2^{mm} above its insertion on the stem, showing cambium. ×150.

FIG. 12. *Erianthus Ravennae;* bundle from the stem a short distance above a node. ×150.



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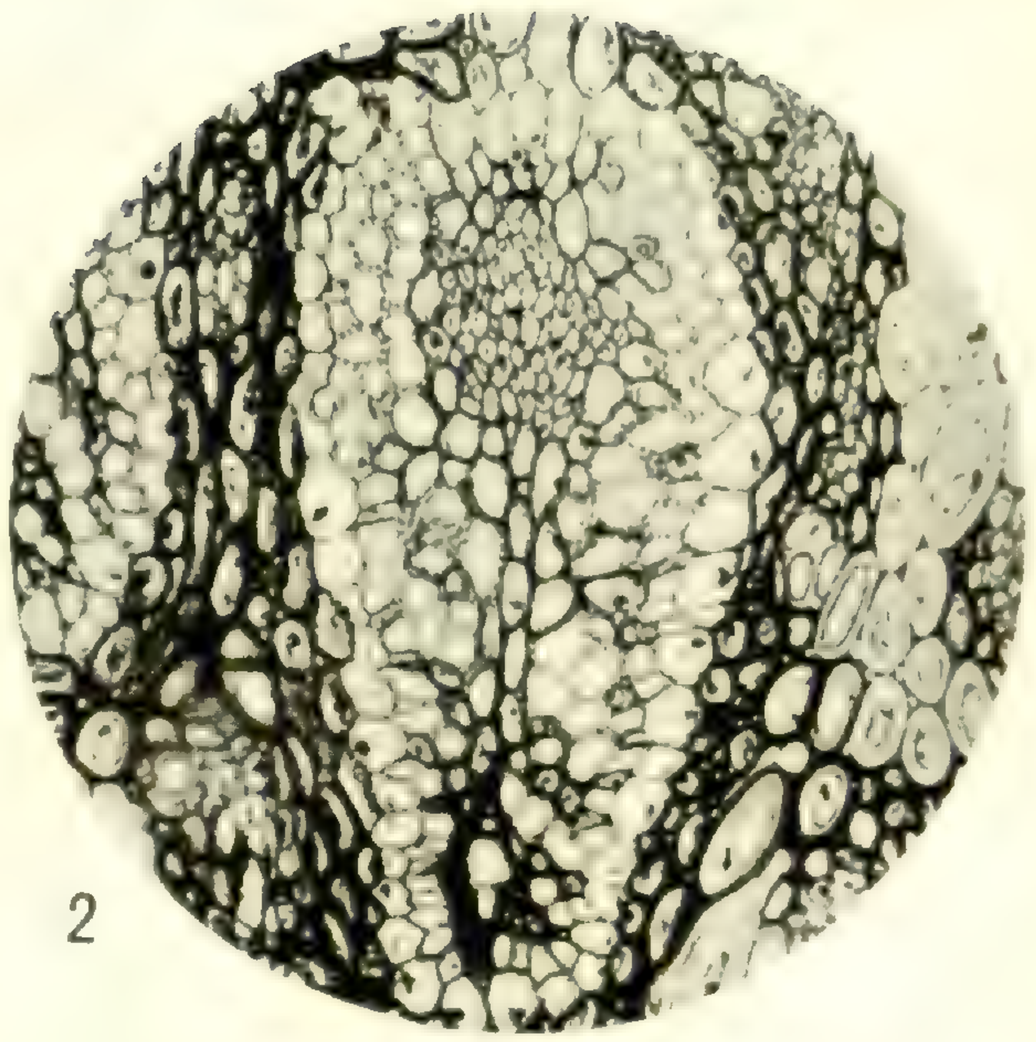

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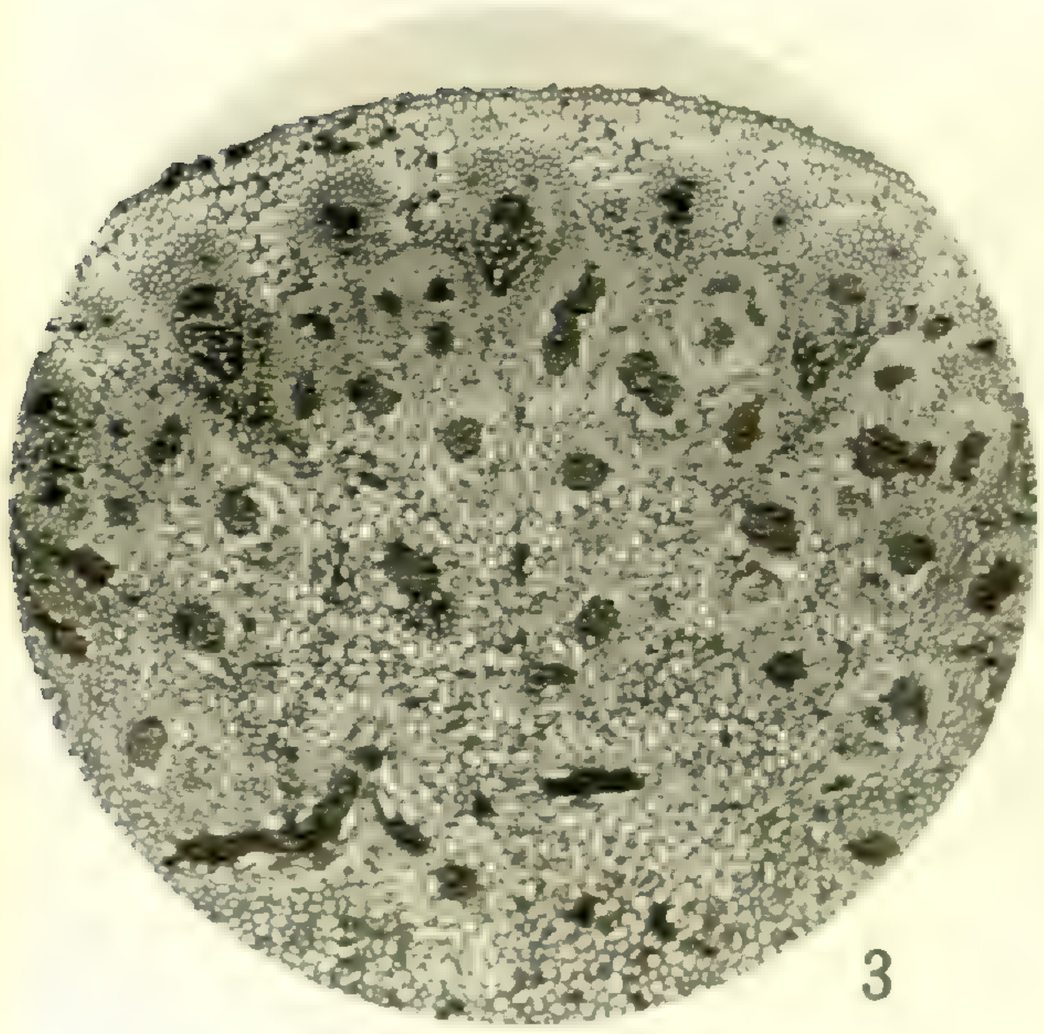

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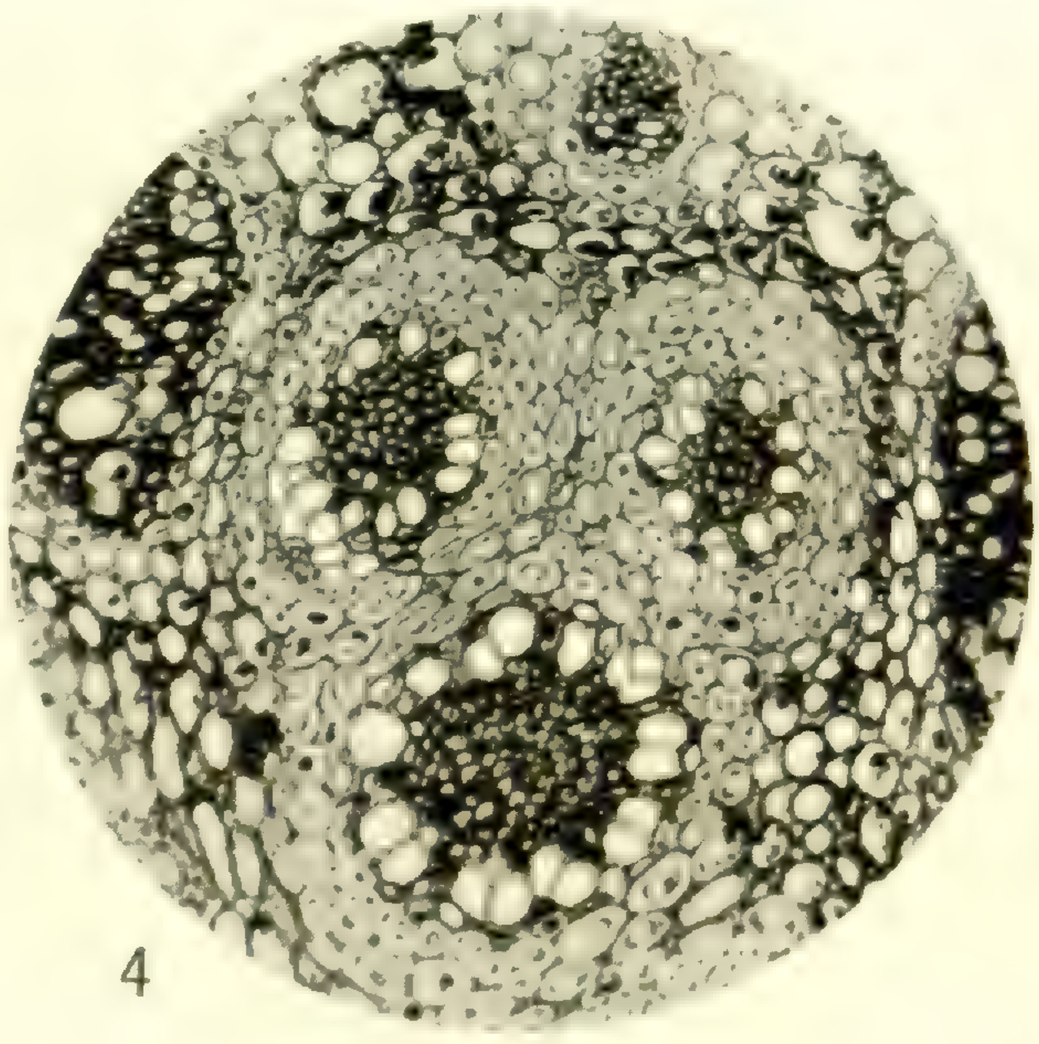

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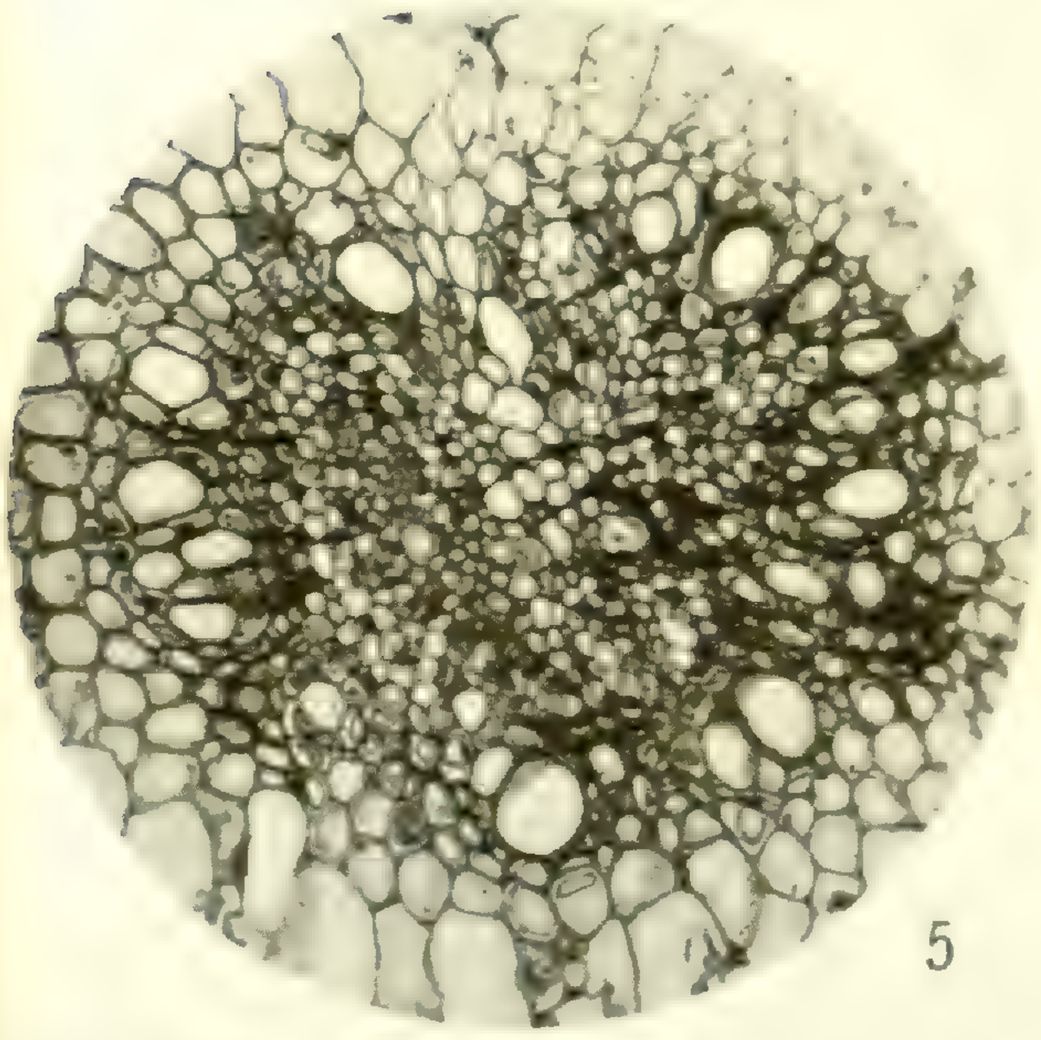

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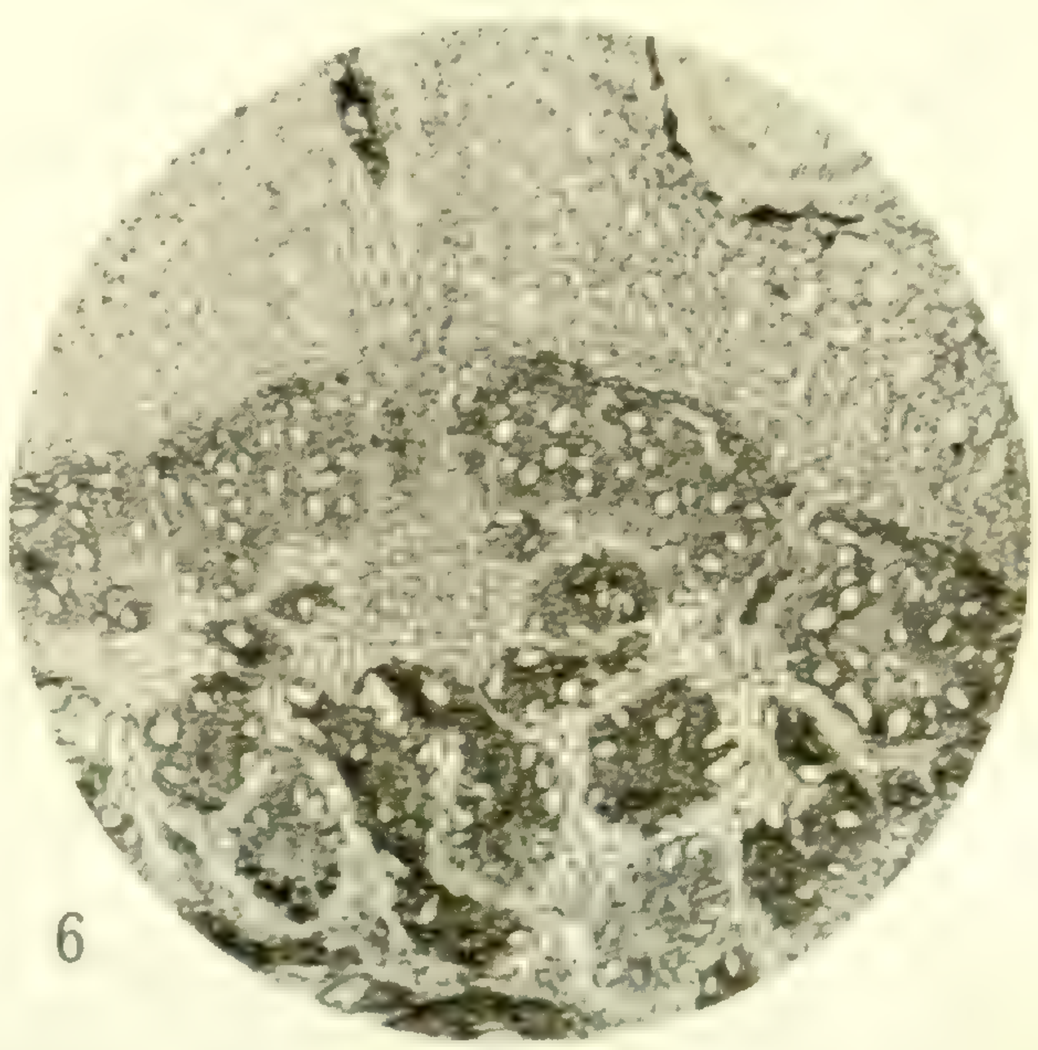

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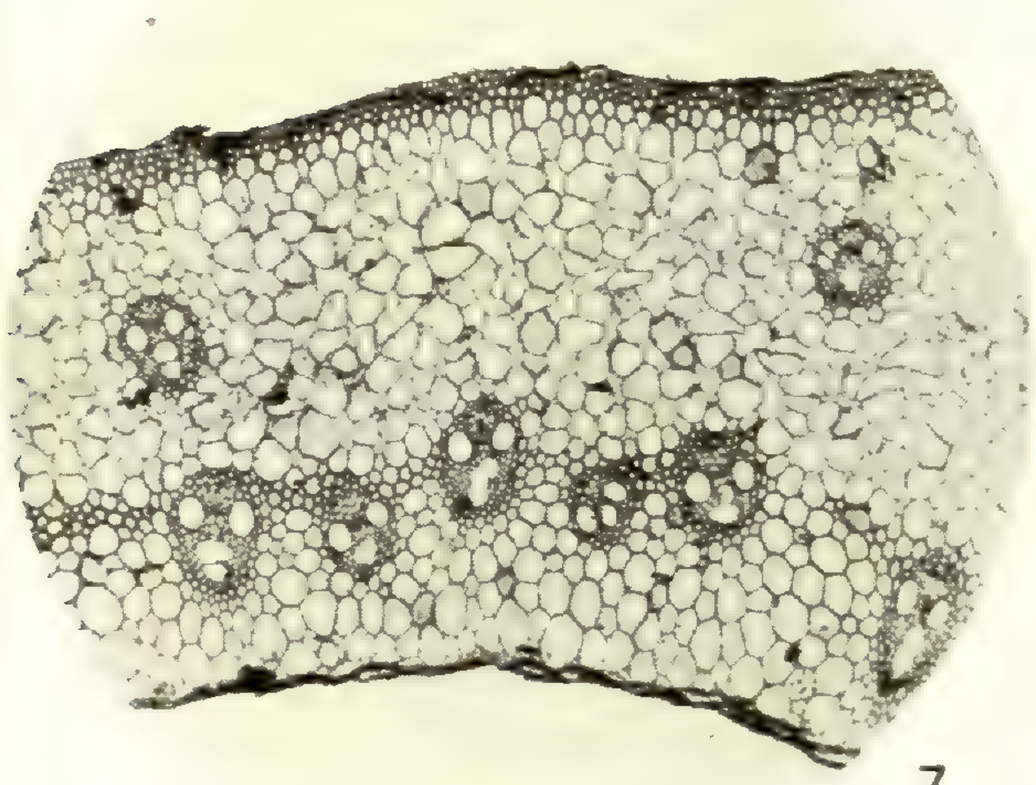
M. A. C. photo.

CHRYSLER on NODES OF GRASSES



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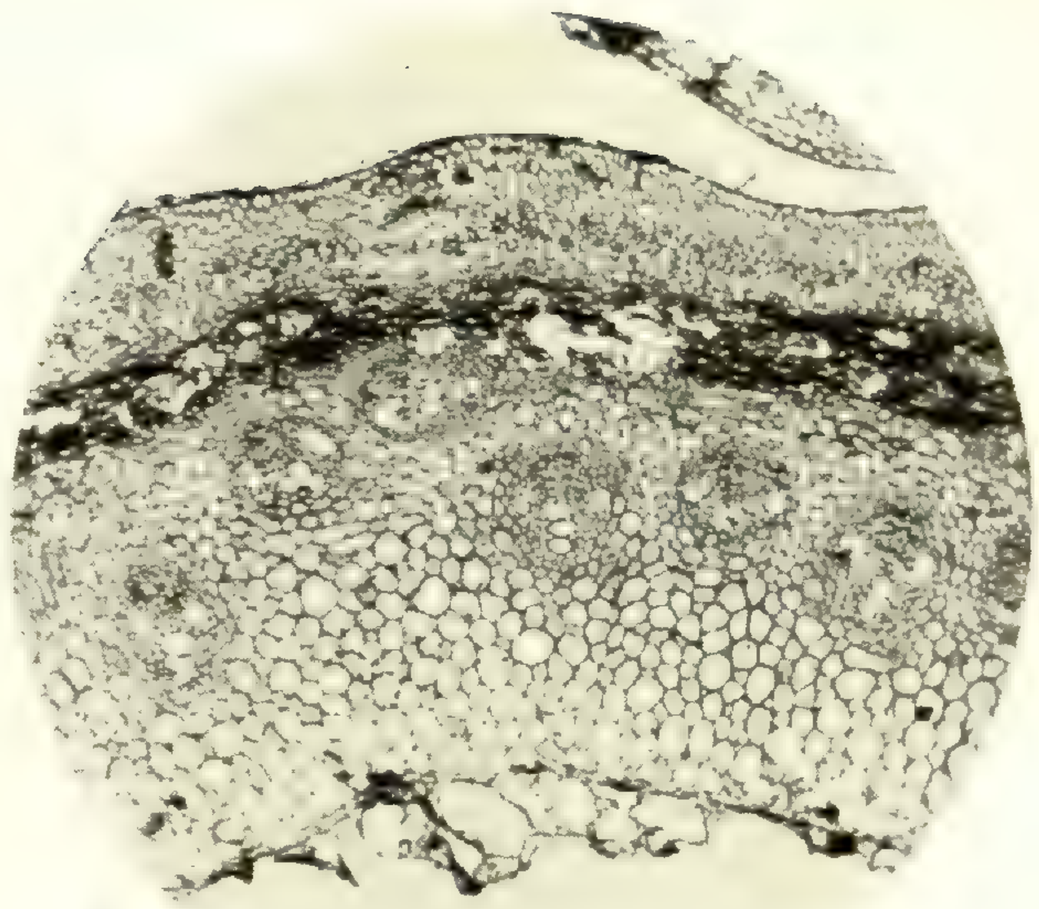

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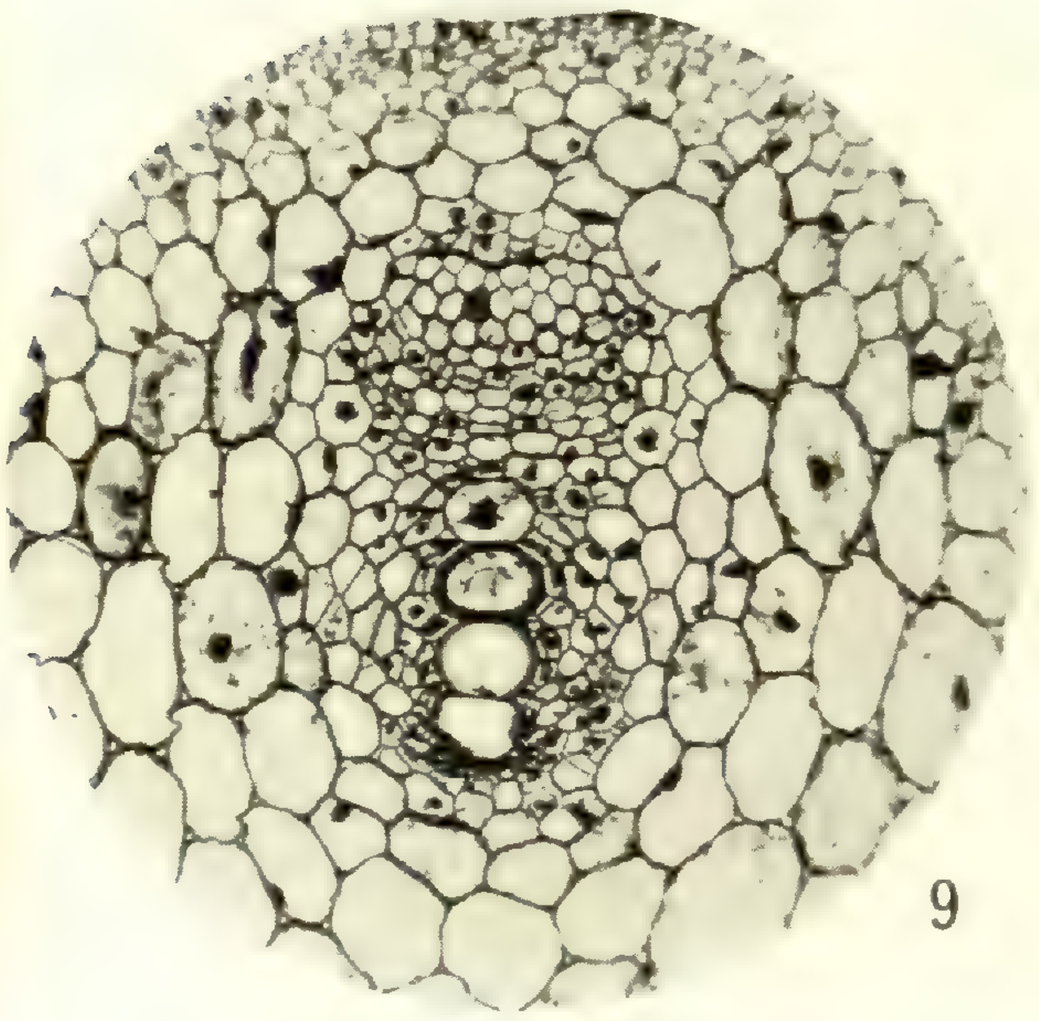

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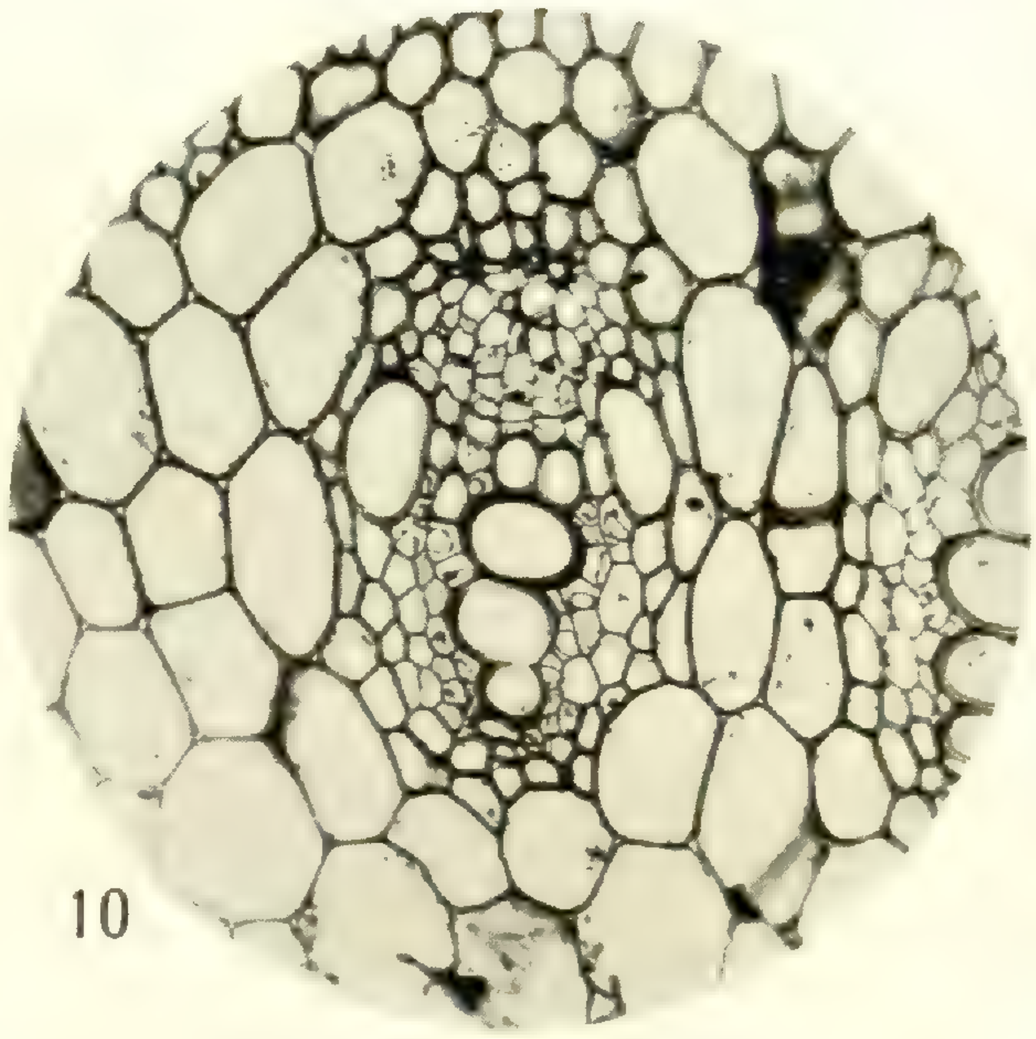

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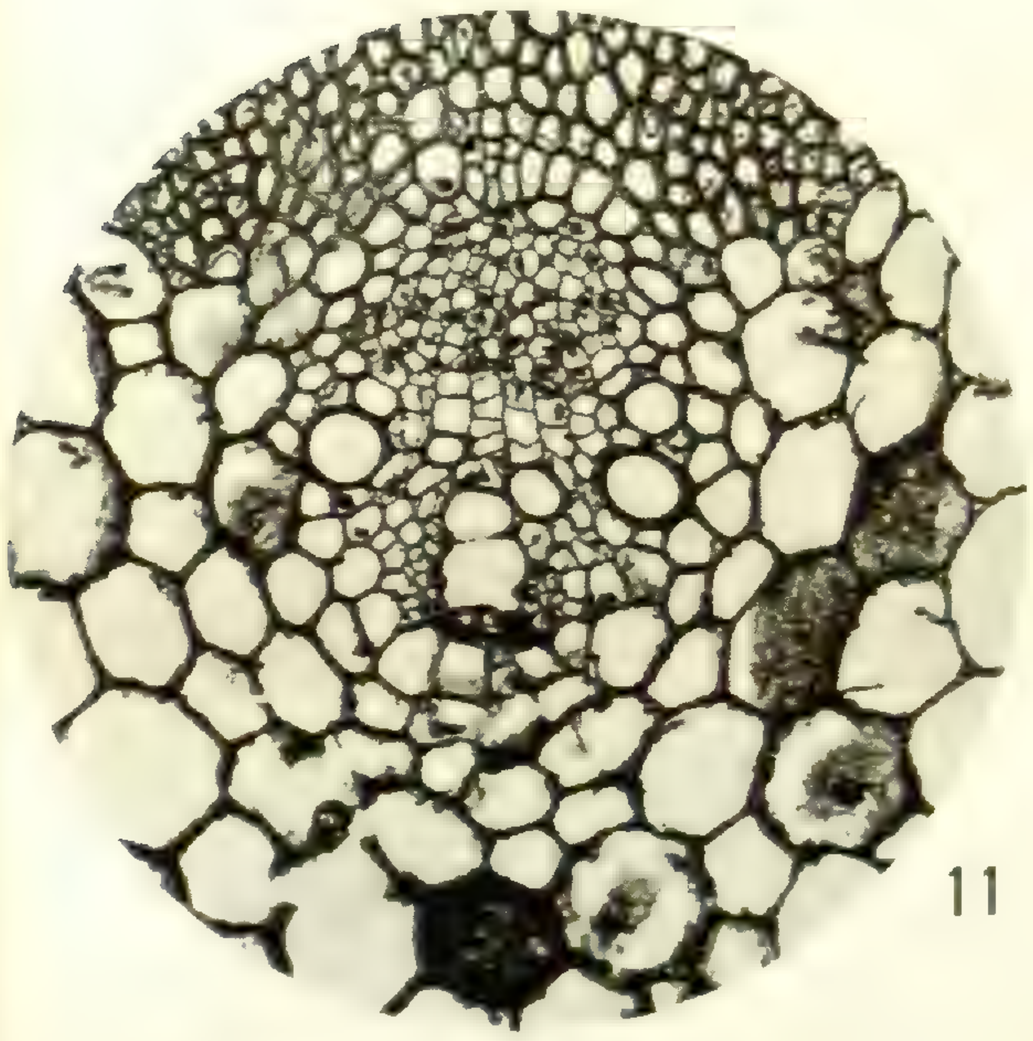

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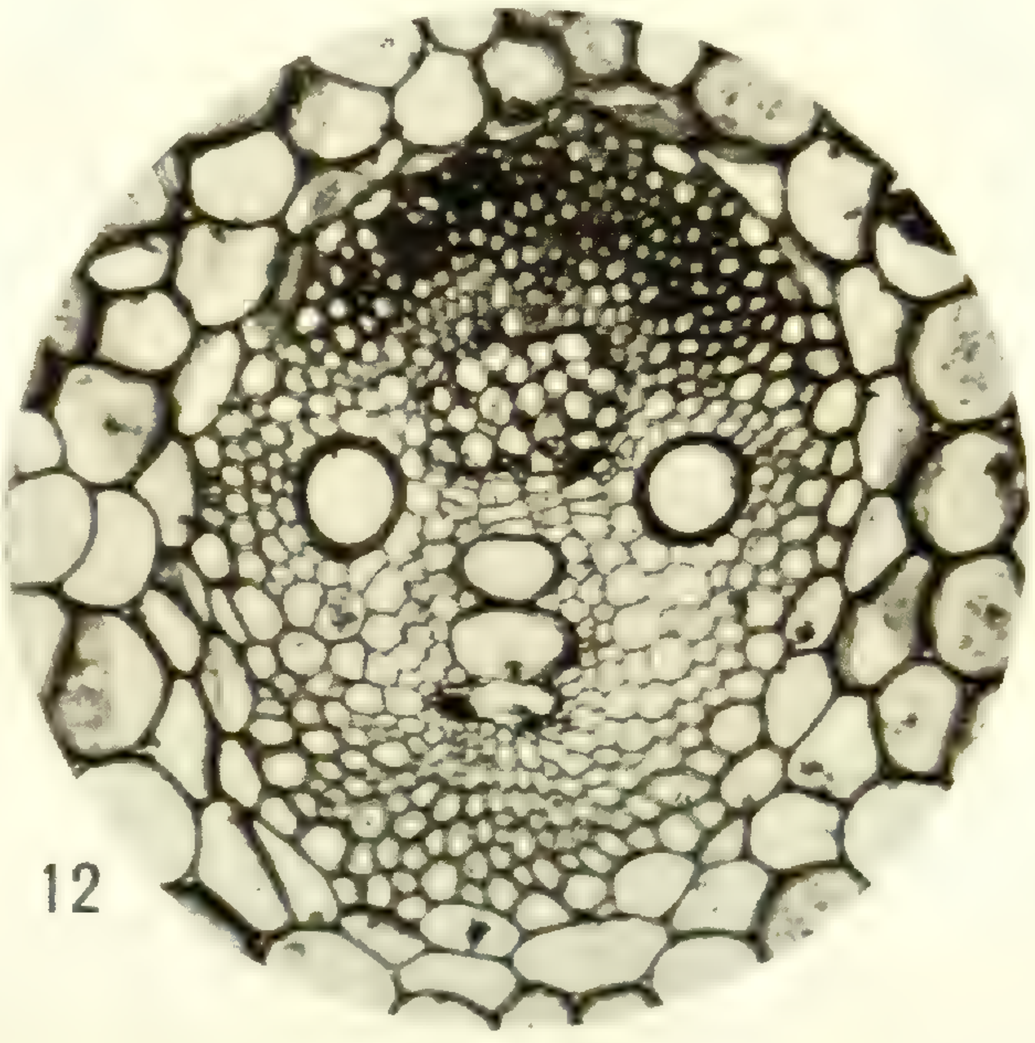

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CHRYSLER on NODES OF GRASSES

THE BOGS AND BOG FLORA OF THE HURON RIVER VALLEY.

EDGAR NELSON TRANSEAU.

(WITH SIXTEEN FIGURES)

[*Concluded from p. 448.*]

IV. The ecological characteristics of the bog flora and their causes.

The plants occurring in the bog habitat are almost all perennials. In the case of the herbaceous vegetation, the winter is passed by means of subterranean rootstocks. The shrubs are in part evergreen and in part deciduous. The tamaracks and the two birches are deciduous, and the black spruce and pine are evergreen.

Most of the herbaceous and shrubby forms multiply abundantly by vegetative shoots of one form or another. The length of the underground stems of the shrubs is proverbial, but is best appreciated by one who has attempted to dig up one of them entire. In connection with the competition between species for space in the habitat, this is of the greatest importance. A luxuriant growth of cassandra furnishes the most favorable situation for the development of sphagnum in this vicinity. Its profuse branching affords a framework for the upbuilding of the sphagnous layer, its shade properties do not interfere with the photosynthetic work of the moss, and it protects it from the drying effects of wind and direct insolation. Where such associations occur, the difficulties presented for the germination for most seeds, and the efficiency with which competition is combated, are evidenced by the fact that among the tree species only the tamarack, spruce, and pine are successful invaders. All of these plants send out adventitious roots from the stems and branches, and so keep pace with the upward development of the moss. The absence of poplars, willows, red maples, and elms in such *undisturbed* situations must be in part attributed to the completeness with which such territory is controlled by the cassandra-sphagnum association.

ECOLOGICAL ANATOMY.

Aside from the purely aquatic forms which have received much

ecological attention, it is of interest to look at the anatomical characteristics of certain of these plants.

Eriophorum virginicum may be taken as a type of this group, and also of the sedge zone vegetation in general. The culm is very slender and erect, leaves flat, and very narrow, perennial by root stocks. *Stem:* epidermis very thick-walled and cuticularized. As development proceeds, certain radial rows of the primary cortex cells have their walls thickened, and served to connect the tissues of the central cylinder with those of the three or four outer layers of hypodermal cells which also become thick-walled. Between these radial groups of cells lysigenic air cavities are formed. *Root:* epidermal cells in part thin-walled and in part secondarily thickened, no definite arrangement of the thick-walled cells apparent; internal structures closely resemble those of the stem; no mycorhiza present. *Leaf:* outer epidermal cell walls strongly thickened and cuticularized, radial and inner walls less so; lysigenic air spaces traverse the leaf longitudinally; a very thick layer of stereome adjoins the leptome, decreasing to one or two cell layers on the hadrome side of the bundle; chloroplasts massed among the outer layers of the cortex, but occur throughout.

Sarracenia purpurea.—Well known for its insect-capturing pitchers. *Stem:* epidermis and first hypodermal layer thick-walled; lysigenic air cavities throughout pith and cortex; resin deposits confined to the epidermis and one or two hypodermal cell layers, but where wounded heavy deposits of resin take place in the exposed and underlying cells. *Root:* cell walls firm, resinous bodies present throughout, but especially prominent in the two outer cortical layers, in which the cell walls are also strongly thickened. *Leaf:* epidermis thick-walled and slightly cuticularized; stomata on both sides of the lamina, with guard cells strongly cuticularized and slightly protuberant; resinous deposits throughout; inner face of lamina with strong downward pointing bristles.

Oxycoccus macrocarpus.—Stem: pith thick-walled, with resinous bodies; a thick layer of broad-celled bast forms a complete cylinder within the epidermis. *Leaf:* margins revolute, upper epidermis without stomata, heavily cuticularized, radial walls thick, wavy; hypodermal collenchyma of two or three cell layers on leptome side

of midvein, one or two cell layers on the side of the hadrome, development of the stereome cells also smaller on hadrome side; palisade of two cell layers; lower epidermis covered with wax, especially at the stomata, guard cells slightly elevated. Mycorhiza present in the larger roots, wanting in the hairlike branches, no root hairs.

Andromeda polifolia.—Leaf: margins revolute, upper epidermal cells thick-walled, radial walls undulate, no stomata; lower epidermis supplied with unicellular short stiff hairs, and covered with wax, stomata slightly protuberant, strongly cuticularized beneath midvein; palisade of three layers of long narrow cells; stereome strongly developed above and below vascular bundle; on the ventral side this adjoining three layers of large thin-walled air cells and a one-layered hypoderma. *Root:* resinous deposits throughout, no mycorhizal fungi found.

Chamaedaphne calyculata.—Leaf: margin slightly revolute, epidermis thick-walled, heavily cuticularized, cuticle rough, no stomata on upper surface; ventral epidermis covered by shield-shaped multicellular hairs, and a deposit of wax; cuticle unusually thickened beneath the midvein, guard cells sunken, subsidiary cells protuberantr palisade tissue of four or five layers. *Root:* inner and radial walls thickened, cortical tissues thick-walled; resin deposits in vascular bundle and cortex; no mycorhizal fungi found.

Chiogenes hispidula.—Leaf: margin revolute, epidermal walls very thick, cuticle present, papillate, palisade not strongly developed; mesophyll cells in part thick-walled and in part thin-walled; resinous bodies in the epidermis; stomata slightly protuberant. *Stem:* resin present in cortex; mycorhizal fungi in the epidermis of the smalle; roots and throughout the cortex of the larger.

Vaccinium corymbosum.—Leaf: cuticle present, epidermal walls not thickened, palisade of one layer, mesophyll tissues with resinous bodies, cuticle of ventral surface papillate; abundant unicellular hairs on lower epidermis few on upper; leptome side of mid-vein adjoined by three layers of stereome and two or three layers of hypodermal collenchyma, on the hadrome side reduced to two of stereome and two of collenchyma, cuticular papilli usually developed beneath the midvein and at edge of leaf. *Root:* cortical tissue with resin, mycorhiza present. No resin deposits found in stem.

Salix sericea.—Leaf: upper epidermal cells small, strongly cuticularized; mesophyll compact, palisade of two layers of long narrow cells; stomata on under surface, guard cells sunken beneath the slightly protuberant companion cells; hypoderma of five- or six-cell layers on hadrome side, and eight layers on leptome side of midvein. *Root:* resinous bodies present in medullary rays and cortex, the latter consisting of thick-walled cells; no mycorhiza.

Ledum groenlandicum.—Leaf: upper epidermis rugose, with scattered unicellular hairs, margins strongly revolute, cuticle present, cell walls thickened, the radial walls being broadly undulate; lower epidermis covered with a thick cuticle and a felt of long multicellular and short unicellular hairs, glandular hairs usually present near the small veins, stomata protuberant; palisade of three or four layers of broadly oblong cells; beneath vascular tissue of midvein and between the mestome bundles occur large air cells which may form lysigenic air cavities in the older leaves. *Root:* mycorhizal.

Larix laricina.—Leaf: bifacial, deciduous; epidermis thick-walled, slightly cuticularized, guard cells sunken beneath the companion cells; palisade tissue developed toward the dorsal surface, two layers thick showing a radial tendency, stereome reduced to a few cells beneath the leptome; two resin ducts near edges of leaf. *Root:* composed of mycorhiza, resinous deposits throughout, cortical tissues early destroyed by fungus. When grown in culture solutions and well aerated, normal roots with root hairs are produced.

Picea Mariana.—Plants in bogs are stunted. *Leaf:* epidermal cells thick-walled, cuticle present, guard cells sunk beneath the companion cells; mesophyll cells compact, of a more or less radial palisade type. *Root:* mycorhizal, resin deposits throughout, cortical tissues destroyed by fungus. Normal roots are developed under culture conditions.

Pinus Strobus.—Plants very much stunted in the bogs, leaves shorter and thicker. *Leaf*, epidermal walls so greatly thickened that scarcely a lumen remains, beneath this a hypodermal layer of thick-walled cells; mesophyll cells compact and of the usual lobate type. *Root:* mycorhizal, cortical tissues traversed by the fungus hyphae; resinous deposits throughout. *Stem:* annual rings narrow

and distorted, resin bodies throughout cortex and meristematic tissues of the wood.

To summarize these characteristics, it is evident (1) that epidermal and hypodermal tissues are thick-walled; (2) that for the conservation of water these are reinforced outwardly by a heavy cuticle, by coverings of wax and air containing hairs; (3) that resinous bodies are found in the roots and leaves of many of the plants; (4) that there is a general reduction in the size of the leaves, and that these are frequently revolute-margined; (5) that palisade tissue is quite uniformly developed; (6) that mycorhizal fungi are present in the roots of most of the plants; (7) that, when compared with the xerophytes of dry sand plains (**25, 6**), they show a similarity in respect to the reduction in size of the foliage, in the development of external protective coverings of the sub-aerial parts, and in the presence of palisade tissues, but are very different in the matter of root development and character of root structures.

To account for the peculiarities of the bog vegetation various theories have been brought forward. KIHLMAN (**28**), in accounting for the xerophilous character of the plants of arctic swamps, which include several species common to American bogs, lays stress upon two factors: (1) the low temperature of the moist substratum, and (2) the presence of drying winds. The former influences the plants by decreasing the power of absorption, the latter increases the rate of transpiration. The plants of such habitats must therefore be protected against the loss of water by the subaerial parts.

SCHIMPER (**44,** p. 11) in classifying the natural habitats in which xerophytes occur mentions among others "peat bogs, because of the humous acids in the soil." On page 18 he says:

> The xerophilous character of the vegetation of peat moors has hitherto been considered an incomprehensible anomaly, and yet the rich supply of humous acids in the soil furnishes a condition for its occurrence as comprehensible as it is necessary. The presence of Scotch pine and heather on both dry sand and on wet peat is thus not more remarkable than is that of *Ledum palustre*, *Vaccinium uliginosum*, and other peat-plants on the cold dry soil in the polar zones.

Further (p. 124) the statement occurs that "on the very acid humus of moors the vegetation assumes a decidedly xerophilous character, because the humous acids impede the absorption of water by the

roots." However, in describing the arctic vegetation (**44,** pp. 11, 715), he follows the suggestion of KIHLMAN, a conclusion to which he had come independently. GANONG (**16**) also accepts KIHLMAN'S explanation for the xerophilous nature of the raised-bog flora of New Brunswick.

In the study of the structural adaptations of these plants and the causes of their occurrence in bog areas, several questions arise. Are these two factors, cold substratum and acidity, efficient causes of xerophily? Do they act, in the case of the bogs of this region, with sufficient strength to cause xerophilous modifications in the plants there found, or to permit the growth of only such forms as are xerophilous?

The last question may be answered from field observations. They indicate that most low-ground plants grow quite as well on the bog substratum as on the ordinary swamp soils, and that the swamp species of this vicinity may all be found at one place or another growing on bog soils. It would seem that here the bog substratum is no more efficient as a selective agent than are the swamp soils.

The only cases which have come under my observation in southern Michigan which will throw light upon the question of the effectiveness of the temperatures and acidity in the production of xerophilous adaptations is in the case of *Picea Mariana*[7] and *Pinus Strobus*. These two plants both show reduced size of stem and leaf, in the Oxford bog, when compared with plants growing on the margin. But to what extent this may be due to sterility of the bog substratum rather than to temperature and acidity is indeterminable at this time.

EXPERIMENTS.

To answer the question of the efficiency of a cold substratum and soil acidity to produce xerophily, experiments have been in progress for approximately two years. The difficulties in the way of experimentation along these lines are numerous. The means for controlling soil temperatures in bodies of soil sufficiently large for experimentation with the larger bog plants are practically beyond the possibility of a university laboratory. When it is further realized

[7] The so-called *P. brevifolia* Pk. This form is certainly no more deserving of a distinctive name than is the bog form of the white pine.

that the experiments should extend over a series of years in the case of the shrubby forms, the problem becomes still more complicated.

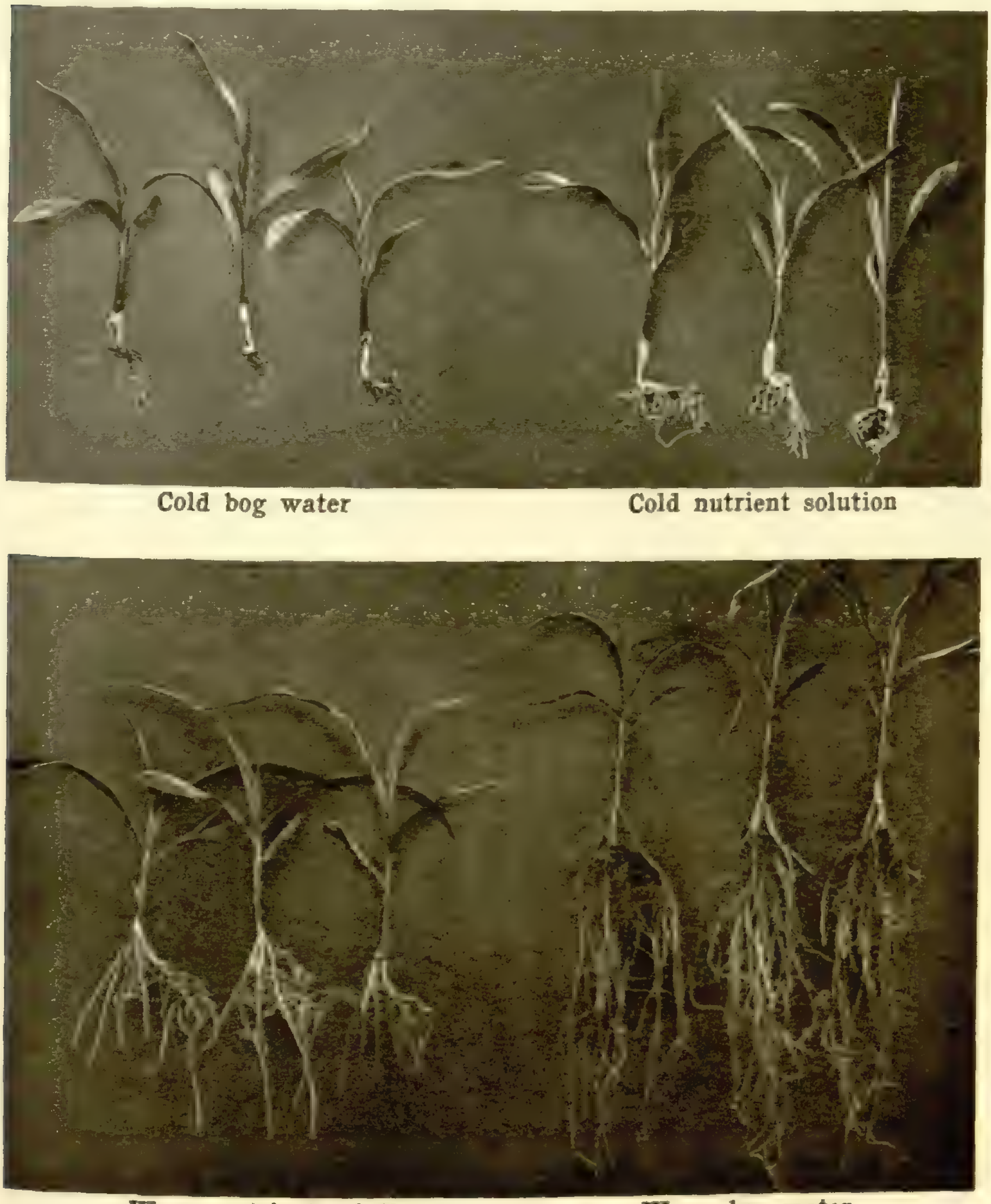


FIG. 12.—Average plants from the several cultures of Indian corn. From photographs.

In order to test the relative effects of humous acids (of the concentration found in the bogs of this vicinity) and low substratum temperatures, experiments were made in the form of water cultures

and with a peat substratum. All of the bog water used was brought to the plant house from the First Sister Lake. The acidity of the water varied from .0005 to .0023 normal acid, as measured by *n* / 100 KOH solution.

WATER CULTURES.—(1) The plants were grown in four-liter battery jars covered with a plaster of Paris plate, having five one-inch openings for the passage of the plants and one of smaller size for a thermometer. Four such jars were employed in each experiment, two containing a 0.2 per cent. Knop's solution, and the others bog water. One of each was further maintained at a lower temperature. The cooling was accomplished by passing tap water through 15 feet of quarter-inch ($4.5^{m} \times 7^{mm}$) glass tubing, arranged in a coil within the jar, somewhat below the surface of the liquid. The sides and bottoms of the jars were covered with black paper, and those which were to be cooled were further surrounded by white paper and sphagnum. Daily readings of the temperatures of the air, warm-water solutions and cold-water solutions during the warmest period of the day were recorded. In this way the maximum differences between substrata and air were obtained. As these temperatures were not constant they exaggerate, to a slight degree, the average differences in temperature. Thus, four conditions were obtained which are comparable: (1) warm nutrient solution (temperature approximating that of the air of the plant-house), (2) warm bog solution, (3) cold nutrient solution, and (4) cold bog solution.

Fig. 12 shows the results of one of these experiments with corn. The photograph was taken eighteen days after the experiment was started. When the cultures were set up, the plumule had developed to a length of 2 inches (5^{cm}). The air temperatures during the period of experimentation averaged 18.8° C., that of the warm cultures 18.7° C., and of the cold cultures 10.8° C.

It is to be noted that under these conditions the best growth of the leaves and roots occurred in the bog water. But a reduction of 8° in the substratum temperatures caused a diminution in the development of both leaves and roots; the plants in the nutrient solution and the bog water being equally affected. When all of the plants had developed five leaves, it was noted that in the case of the cold cultures the two lower leaves had withered. This experiment was

repeated with corn, white lupine, and bean under similar conditions, with similar results. The greater development of roots in the case of the warm bog water may be due to the presence of a poison in very minute quantities; but this I have been unable to prove.

(2) A third culture was then made in which five plants of corn were grown in each of the four water culture conditions, and in addition in four similar conditions, using a mixture of sphagnum and peat for the substratum. Wooden boxes 2 feet long, 1 foot wide and a half foot deep ($60\times30\times15^{cm}$) were constructed, and two were lined with galvanized iron. The bottoms of the unlined ones were perforated so as to allow of easy drainage. The lined boxes served for the undrained conditions. Further, in one of the drained and in one of the undrained boxes, 40 feet (12^{m}) of glass tubing, bent into coils, the joints being connected by rubber tubing,were arranged so that a constant flow of cold water, for lowering the temperature, could be maintained. The water level in the undrained bog substratum was kept just below the surface. The water was obtained from the bog at First Sister Lake, but occasionally all were watered with distilled water. The amount added to each box was practically the same. In order to keep the solutions in the water culture jars at the same acidity as in the undrained boxes, the water was siphoned off and transferred once a week. Care was taken in this transfer to aerate the water in the boxes as little as possible, while that of the jars was aerated at irregular intervals by means of a bulb. There were thus produced eight conditions, in which it was possible to test the effect of the acidity of the bog water, of aeration (drainage) of the substratum, and of low temperatures. As a result, it was found that the growth of roots and leaves was best in the warm bog water, in the warm nutrient solution, and in the drained warm peat substratum. Reduction in size of both roots and leaves occurred in the cold bog and nutrient solutions, and in the drained cold and undrained warm and cold peat substrata. But the plants in the undrained cold peat showed the most marked reduction in size. The conclusion was reached (1) that humous acids (acidity varying from .0005 to .0023 normal acid) have no effect upon corn in the matter of leaf and root development; (2) that low temperature and lack of aeration of the substratum both cause reduction in size;

and (3) that when low temperature is combined with poor aeration the effect is very marked.

This experiment was repeated with peas, and the same result was obtained, although the effects were not so marked (*fig. 13*). The roots in the undrained substrata were killed when they attained a depth of a half inch (12^{mm}) below the surface.

(3) In order to test the effects of drainage and of low temperature on bog species, another set of cultures in peat-sphagnum substrata was made. The apparatus used consisted of two flower-pots and two glass dishes aproximately a foot in diameter and three inches

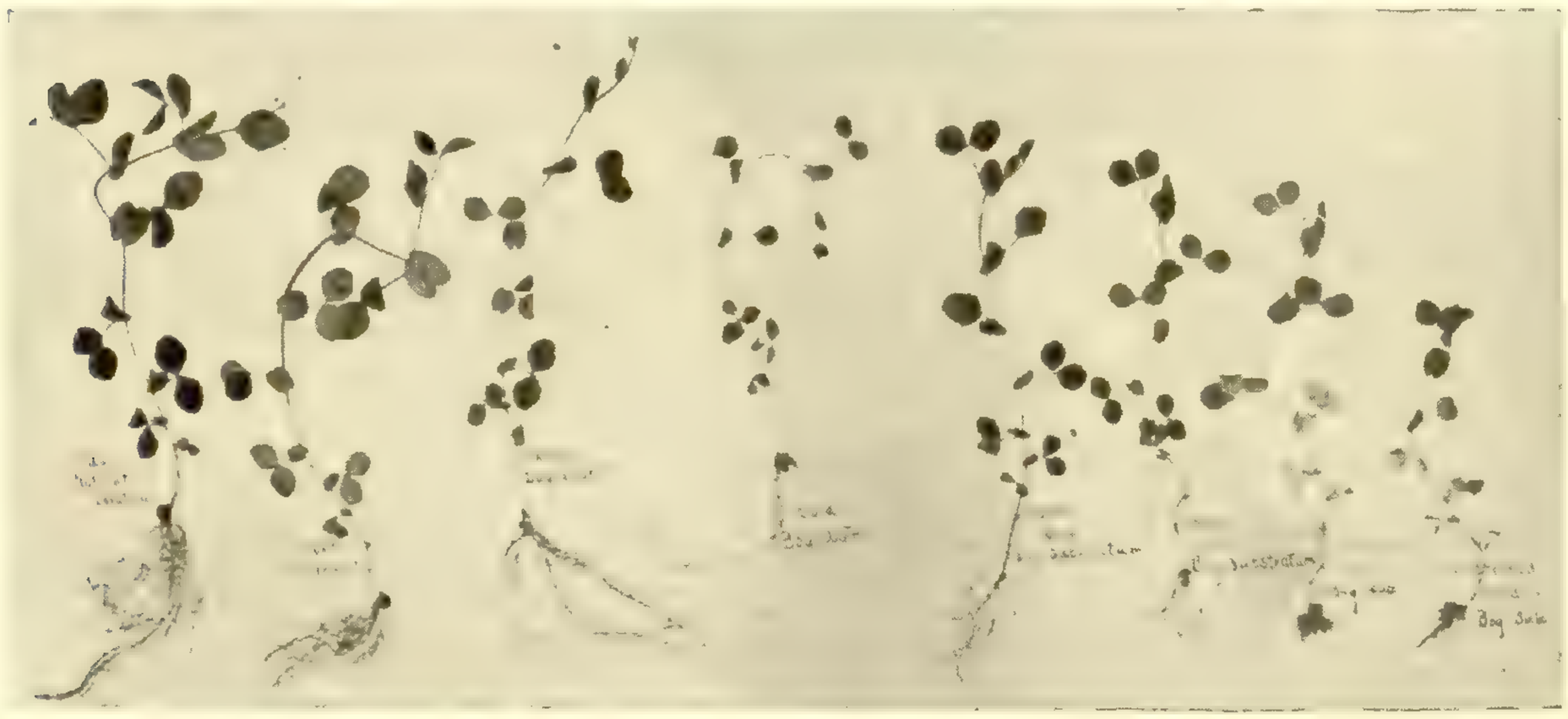
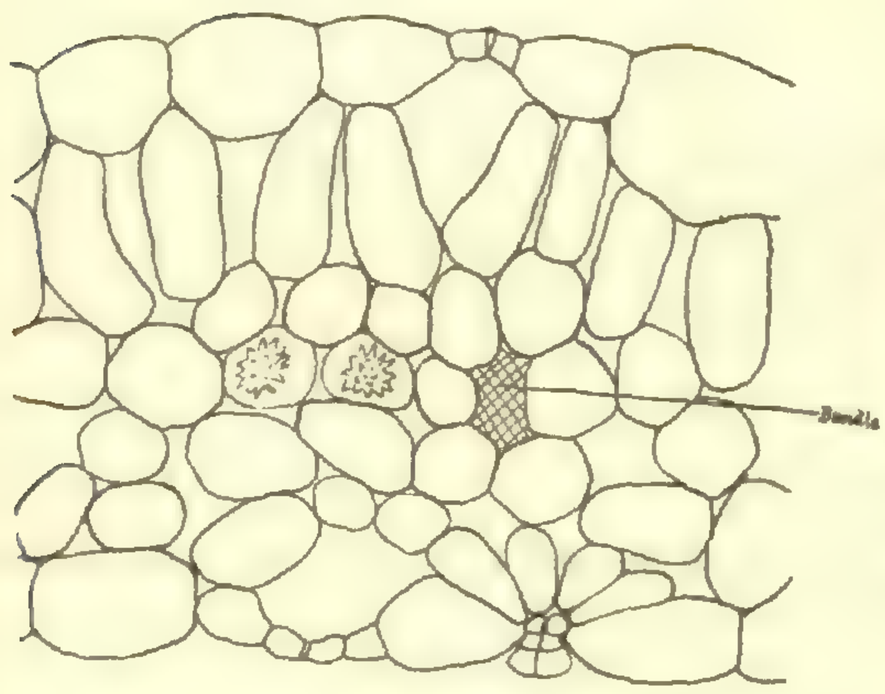


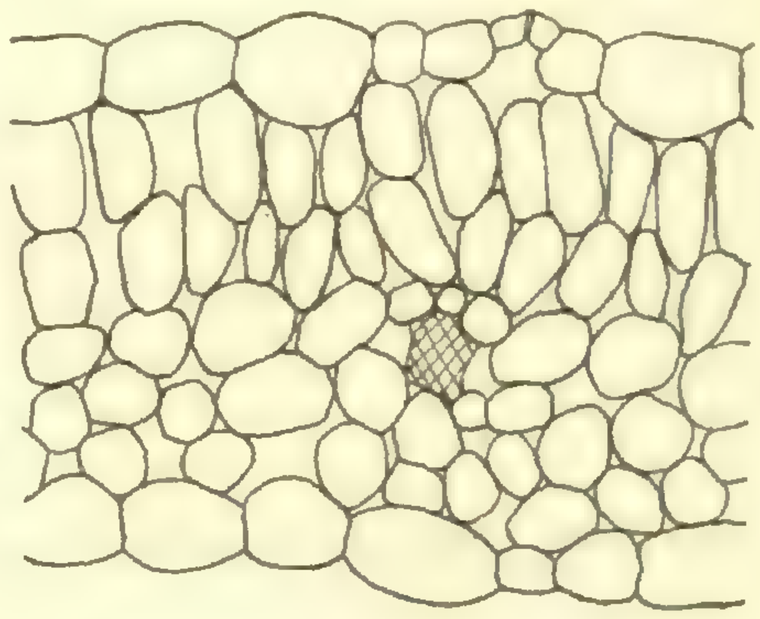
FIG. 13.—Effect of the several conditions upon the development of pea seedlings. All are average specimens. From photographs.

deep (30×7.5^{cm}). A flower-pot and a glass dish were kept cool by passing cold water through fifteen feet of glass tubing arranged in coils, as in previous experiments. Three species were tested in these conditions: two-year-old Larix laricina, *Rumex acetosella*, and *Prunella vulgaris*. The first cultures were made in the spring of 1903 with the Rumex and Prunella. The air temperature averaged about eighteen degrees. The cold substratum was maintained about ten degrees lower. In the case of Rumex it was found that the largest leaves were produced in the drained peat-sphagnum substratum. Lack of drainage and low temperature both caused a reduction in leaf area, and when combined produced leaves which were less than half as large as those of the drained warm substratum.

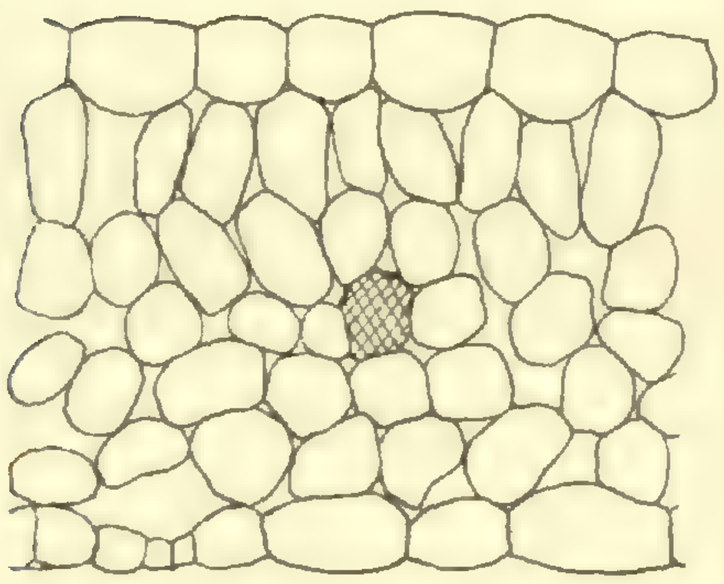
The Prunella under the same conditions showed the same results.



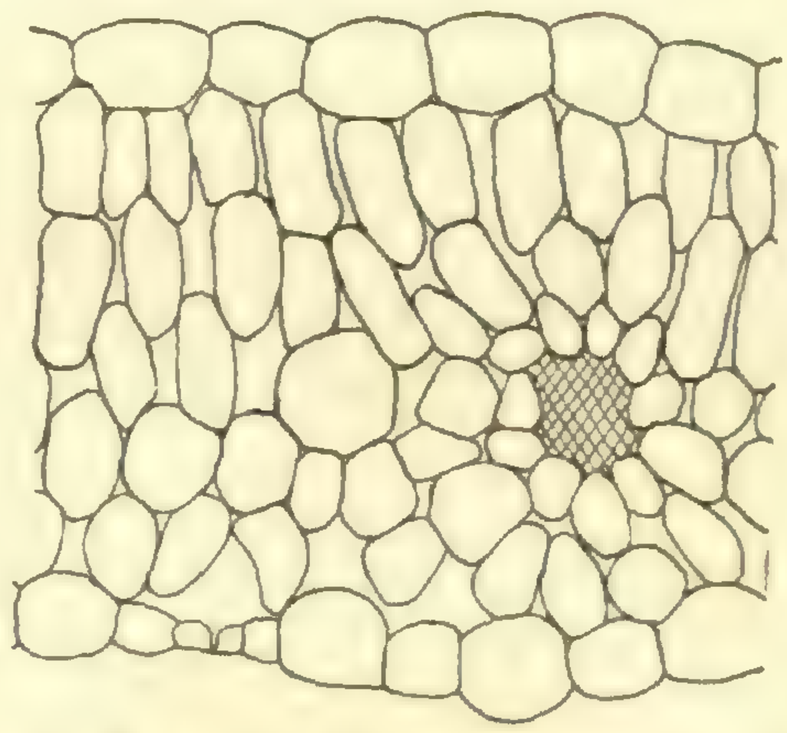
A. Drained warm bog substratum



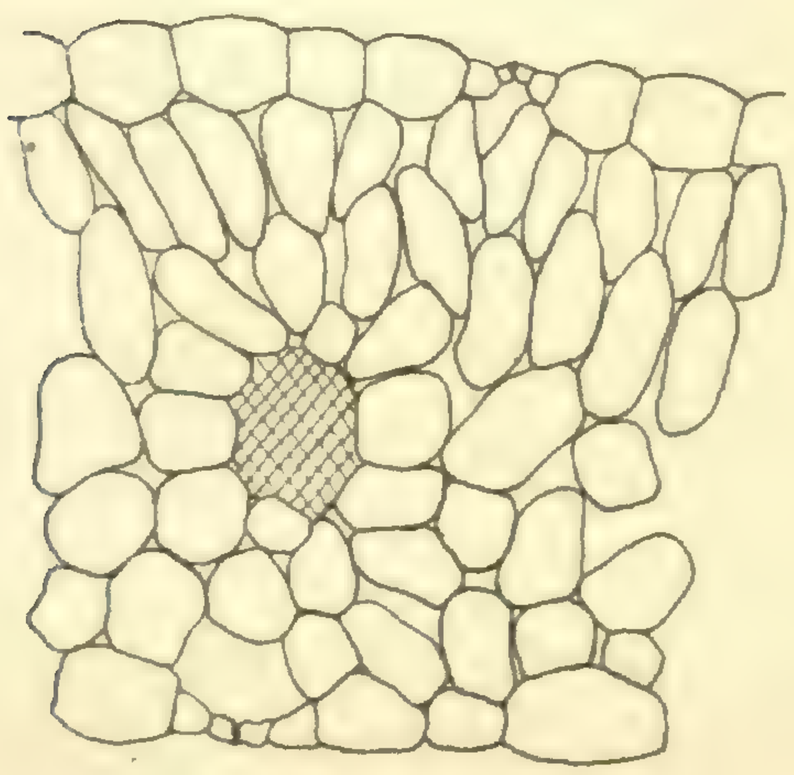
B. Drained cold bog substratum



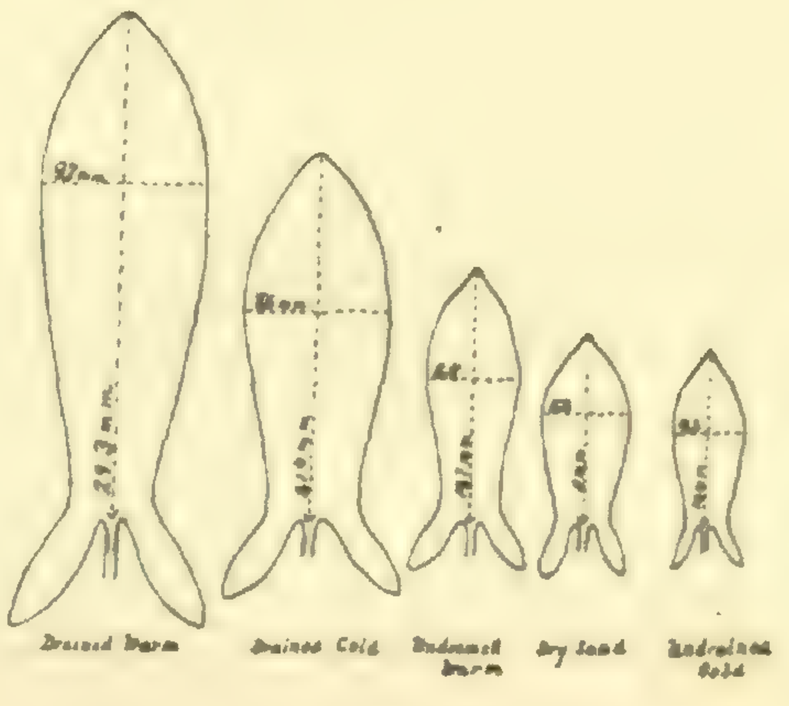
C. Undrained warm bog substratum



D. Undrained cold bog substratum



E. Dry sand



F.

FIG. 14.—*A*, *B*, *C*, *D*, *E*, camera drawings of leaf sections resulting from cultures in the five conditions named. ×135. *F*, diagrams showing average length and breadth of leaves.

Fifteen plants were grown in each condition. At the end of the experiment each had produced six to eight mature leaves. The leaves were measured as to length and breadth. An index was obtained by multiplying these two numbers together and averaging for each culture. Following are the indexes of leaf area thus derived: drained warm substratum 1268.3, drained cold 682.6, undrained warm 518.5, undrained cold 421.8.

In the spring of 1904 the experiment with Rumex was repeated. The results correspond with those of the preceding year. The structure of the leaves, resulting in the several cultures, was investigated, and found to show marked variations (**56**). *Fig. 14* represents the cross-sections and average leaf areas produced (seventy-five leaves being measured in each case). When grown on a warm drained substratum, the leaves are large, and the cells are exceedingly loose and turgid. The epidermis is composed of large thin-walled cells, having a thin cuticle outside. The mesophyll consists of a single layer of palisade and three layers of spongy tissue. No resin bodies are present. The plants grown in the undrained substratum, whose temperature was reduced about 8° C. below that of the air, show marked xerophilous characters. The leaf is reduced in area, increased in relative thickness, and the margins become revolute; the epidermal cells are smaller and outwardly thick-walled; a well-marked cuticle is present; the mesophyll is very compact and made up of two or three layers of well-developed palisade cells and three layers of spongy tissue; and in the epidermal cells and those adjacent to the bundles there are marked accumulations of resinous bodies.

For the purpose of comparison, a corresponding set of plants were grown on sand kept just sufficiently moist to allow the plants to live. As will be seen in *fig. 14*, the xerophily is not more marked than that of the undrained cold bog substratum. *Fig. 15* shows the relative appearance of the plants produced by the different conditions.

In the case of the plants grown in the undrained warm and the drained cold substrata, these same effects were noticeable, but to a less marked degree. That, in the case of the undrained cultures, these effects are not due to the acidity of the bog water is shown by

the fact that plants grown in bog-water cultures develop normally.

The light conditions in the several cultures were the same, direct sunlight being avoided by a cloth screen. It is evident that in this case there is no response to strong light in the development of the palisade tissue (**49**). It would seem rather to be a response called forth by a reduced transpiration current (**44,** p. 7). As to function, it may aid in the transfer of food materials as suggested by HABERLANDT (**20,** p. 260).

FIG. 15.—Average plants showing effect of surrounding conditions. From photographs.

This plant proved to be the most plastic of all of the species used in the experimentation, and was the only one which showed marked variation in the internal structures. Ecologically the results indicate (1) that an undrained peat substratum may cause xerophilous structures, but that the effect is to be correlated with lack of aeration of the substratum rather than with the acidity; (2) that the same effect may be induced by lowering the substratum temperature (the air temperature remaining the same), and thus impeding the rate of

root growth and absorption; (3) that a cold undrained bog substratum is analogous to a dry warm soil in that both produce physiological drought; (4) that resin bodies, which are characteristic of the bog plants, may be produced by this environment in a plant which under favorable conditions is without them.

FIG. 16.—Relative effects of drainage and reduced substratum temperature, on Larix. From photographs.

The seedling tamaracks, ten of which were cultivated in each of the four conditions just described for the Rumex, also showed considerable variation. Their relative development at the end of forty-

four days is shown in *fig. 16*. The leaves of the drained warm substratum have an average length of 12.6 mm, of the drained cold 10 mm, of the undrained warm 11.4 mm, and of the undrained cold 6.3 mm. Internally, the leaves show a reduction in the intercellular spaces and in the size of the cells in the case of the plants grown on the undrained cold substratum, when compared with those of the warm drained condition.

(4) In another series of experiments with plants of Larix four to five years old practically the same results were obtained. There were the greatest number and length of leaves and branches produced in the case of the drained warm substratum. The smallest and shortest leaves and branches were produced by the undrained cold substratum.

Experiments with *Ledum Groenlandicum*, *Chamaedaphne calyculata*, *Andromeda Polifolia*, *Betula pumila*, and *Oxycoccus macrocarpus* have failed to produce satisfactory results. This is believed to be due to the shortness of the time under which they were under cultivation. The plants were brought from the bogs in the late autumn and placed in cold frames over the winter. About the beginning of March they were brought into the greenhouse, and after a few days planted in the warm and cold, drained and undrained boxes, previously described. They have grown vigorously, but the differences noticeable may not be correlated with the four conditions. The cranberry has shown the greatest amount of plasticity, but this could not in all cases be correlated with the environment. If these plants can be kept under known conditions for two or more years, it is probable that they will yield valuable results.

(5) In order to test the effect of mineral soils, and the ability to withstand the presence of large quantities of calcium and magnesium, specimens of andromeda, cassandra, and cranberry were grown in sandy loam and sand. They were watered daily with tap water. The cultures were started in the autumn of 1902, and produced vigorous vegetative shoots during the summer of 1903. They failed to bloom, however, and although they are growing well at this time (June 1904), they have again failed to bloom. This may be in part due to the warm plant-house conditions. The experiment was originally started to observe the changes in the roots, and in so far

have been of value. In a sphagnum substratum all three of the plants produced hairlike roots which attain a length of 5–7 cm. The roots are commonly several times branched, very little difference in thickness being shown by the several branches. When grown in sand the roots are still slender, but the frequency of branching is enormously increased. Usually the branching occurs just back of the growing tip. The older root ceases growth as the lateral root develops. The branch continues for 2–3 mm, and it also stops growth with the formation of a second lateral root. The result of this process is a zigzag root showing root branches which have been successively the main root tip. Occasionally several lateral roots develop and the main axis is divided.

(6) The statement that waters containing lime and other mineral salts are unfavorable to the growth of sphagnum has gained wide circulation in ecological literature. Because of the great abundance of lime and magnesia in the waters of this vicinity, I was led to test this fact by growing the sphagnum in tap water and solutions of $CaCO_3$. In one experiment the water in a battery jar was saturated with CO_2, $CaCO_3$ was added in excess, and the CO_2 was again allowed to pass through the water for thirty minutes. In this solution sphagnum was placed, and it has been growing vigorously for three months, although watered daily with water containing over 100 parts of $CaCO_3$ to the million. Some of the sphagnum cultures have been running for ten months, and show no signs of deterioration. Whether the sphagnum of this vicinity has become accustomed to the presence of lime, owing to the nature of the soil waters, or whether sphagnum is generally able to withstand such conditions, remains to be proved. Since the above experiments were performed, I have found an account of somewhat similar experiments by WEBER (58), the results of which are the same. It would seem, therefore, that the presence or absence of sphagnum is not to be correlated with the presence or absence of lime.

(7) Among the plants growing in the bogs of this vicinity the following have been found to possess mycorhiza: *Larix laricina*, *Pinus Strobus*, *Picea Mariana*, *Betula lutea*, *Betula pumila*, *Oxycoccus macrocarpus*, *O. Oxycoccus*, *Chiogenes hispidula*, *Vaccinium corymbosum*, *Ledum Groenlandicum*, *Populus tremuloides*.

In order to get at the conditions which favor or cause the development of mycorhiza, cultures of Larix were made in loose sphagnum, sand, undrained sphagnum, etc. The roots in the many other cultures previously noted were also carefully watched. It has been found without exception that where the plants were grown under properly aerated soil conditions, normal roots developed in place of the mycorhiza. That the acidity of the bog water has nothing to do with the production of mycorhiza is shown by the fact that in water cultures of the same acidity as the solution in the undrained peat, the plants develop normal roots. In the case of roots developed in loose sphagnum, sand, and moist air, an abundance of root hairs were produced. The normal roots in Larix have a diameter about three times that of the mycorhiza, so that when they begin to develop they appear like white pendants from the dark brown mycorhiza. That mychoriza will not develop in a well-aerated substratum was further tested by the following experiment: Two 30^{cm} test tubes were set upright, and 8^{cm} of glass beads were poured into the bottom of each. Into one a glass tube, at whose end were several small openings, was passed to the bottom. The upper part of the tube was connected with a gasometer. Upon this foundation of beads, three plants of Larix were planted in a 5^{cm} layer of peat in each tube. The water level in the two tubes was kept just at the surface, bog water being used throughout. Air was then forced from the gasometer to the bottom of the one tube and allowed to pass slowly through the beads and peat. When the experiment was started, all of the plants possessed only mycorhiza. In the course of a week the aerated plants began to develop normal roots. The experiment was continued for six weeks. The unaerated plants developed only mycorhiza, while those which were aerated developed normal roots.[8] The growth of mycorhiza is exceedingly slow, and the fungus grows with the root. The development of the above ground parts corresponds to the root development. The plants which produce normal roots have longer shoots, and longer, thicker leaves.

It seems evident, in the case of Larix at least, that (1) the mycorhizas develop only in poorly aerated substrata; (2) their growth is

[8] In the case of a number of the plants of Larix grown in the undrained peat in previous experiments, one or two normal roots were developed just at the surface of the substratum.

exceedingly slow, the fungus developing along with the root; (3) the acidity of the substratum is not a factor in their development; (4) in a naturally well-aerated soil or in an artifically aerated substratum normal roots develop; (5) when the roots are not surrounded by water, root hairs develop abundantly. Mycorhiza therefore appears to be an abnormal root condition. Whether the fungus is of advantage to the root under these poorly aerated conditions cannot as yet be stated.

(8) In order to determine whether the zone of tamaracks follows the shrub zone because of the occasional submergence of the sedge zone, the following test was made: Ten Larix seedlings averaging 7^{cm} in height were placed in a crystallizing dish with the roots imbedded in 2^{cm} of sphagnum. Over this a layer of bog water 4^{cm} in depth was maintained for six weeks. The plants grew quite as well as those in a peat substratum. Stem and root submergence is therefore not a factor in preventing the growth of seedlings tamarack in the sedge zone. The liability to submergence in the bogs I have studied would not extend over nearly so long a period of time.

V. Summary.

The Huron River basin shows three well-marked physiographic divisions which differ in forest covering and the possibilities for bog development. These are (1) the region of the Saginaw-Erie interlobate moraine; (2) the Erie morainic belt; and (3) the lake plain.

In discussing the meteorological conditions of a region as affecting the flora, attention is called to the fact that the significance of the data is not apparent unless the temperature and rainfall phenomena are compared with those of the optimum region of dispersal of the plant societies involved. In the case of the bog plant societies the temperature of the region under discussion averages several degrees higher during the summer months than the eastern maritime provinces of Canada (the optimum region of dispersal for the bog plants), while the rainfall during the same period averages about three-fourths as much. This is believed to account for the general difference in character and development of bog societies in the two regions.

Bog and lake basins are here associated with deposits of glacial drift. The most frequent causes of these basins are (1) the melt-

ing of stagnant bodies of ice in old glacial drainage channels after their abandonment; (2) the differential settling of fluvio-glacial deposits; and (3) unequal deposition of glacial material in moraines and till plains.

Marl and peat deposits are commonly associated. The former are of interest in so far as they aid in the filling of the lake basins. Both are formed through plant agencies.

Peat deposits may be classified under two general heads: (1) those connected with glaciation, and (2) those associated with coastal plain phenomena. In North America the bulk of the deposits come under the first head. Their geographic distribution approximates that of the Pleistocene glaciers. Near the southern border the peat areas are scattered, but they become more nearly continuous and more independent of depressions as we go northward. The same effect is brought about in mountainous regions by increased altitude. In the tundra, peat accumulates because of the low temperature and in spite of the scant vegetation. In temperate regions a vigorous vegetation and areas of stagnant water render peat accumulation possible. In the southern coastal plain swamps, peat is formed in stagnant water because of the luxuriant vegetation and in spite of the high temperature.

During peat formation two processes are involved: (1) eremacausis and (2) putrefaction. The former is essentially an oxidizing process, brought about in the presence of air by certain fungi and bacteria. Its products are of direct value as food materials for plants. Putrefaction is carried on in the absence of oxygen and is essentially reduction; the organisms involved are anaerobic bacteria, and the products are of no value to the higher plants as food materials. The accumulation of peat depends upon the scarcity of oxygen below the water level, the acidity of the ground water, and the occurrence of low temperatures.

Peat varies in color beneath the various plant societies, being light brown in the youngest (bog sedge) and dark brown in the oldest, the darkest and most thoroughly decayed form being known as "muck." As disintregration proceeds it brings about a decrease in water capacity, a decrease in volatile combustible matter, and an increase in the amount of ash.

The bog as a habitat for plants differs widely from the other plant habitats of the region in that its substratum has been built by forerunners of the present vegetation. Owing to the influence of the wind in the production of waves, the bogs are largely wanting on the eastern shores of lakes, and in the case of basins which have been almost completely filled with peat, the open water lies toward the eastern margin.

It is well known that bog areas are more liable to late spring frosts than adjoining uplands. This is due to the topography as it affects air drainage, and to the low conductivity of the substratum covering. Under natural conditions it has been found that the areas of cassandra and tamarack dominance are more exposed to late frosts than other societies.

Observations in bog areas show that the soil temperatures beneath the several plant societies differ markedly in range. The records indicate that the areas of bog sedges have temperatures corresponding closely with those of the upland and approximating those of the atmosphere. The willow-sedge (swamp) and maple-poplar areas have slightly lower temperatures during early spring. When the trees leaf out, however, the shade produced causes the maple-poplar area to have the lowest temperatures recorded. The bog shrub and tamarack societies show the lowest average temperature throughout the spring months.

Low soil temperatures retard chemical action, diffusion, solution, and osmosis, and render the substratum unsuited to soil bacteria. When coincident with higher air temperatures, plants having a low transpiration ratio are favored in the competition between species.

In so far as southern Michigan is concerned, the substratum temperatures prevailing in bog areas do not seem to be adequate to account for the presence or absence of bog plants or their xerophilous structures. Experiments suggest, however, that farther north this factor is of prime importance.

In texture the bog substratum shows every gradation from the coarse fibrous peat of the bog-sedge zone to the black powdery muck of cleared land. Bog soils in general do not afford as good a foothold for trees as do the mineral soils.

Peat is very resistant to the diffusion of mineral salts, hence bog

areas have a very different soil solution from that of the mineral soils adjoining. The high water capacity of peat is detrimental to plants, in so far as it prevents proper aeration of the substratum. Bog waters have no higher osmotic pressure than ordinary soil waters.

The absence of sphagnum from local bogs cannot be explained by the presence of calcium salts, as shown by observation, chemical analyses, and experiments.

The acidity of local bog water varies from .00015 to .00258 normal acid. The lowest values are found in areas covered by bog sedges and swamp plants, and they are approximately the same. The highest occur under the tamaracks. The variations in acidity are related inversely to the temperature. As shown by experiment, this is because of increased oxidation at the higher temperatures. It is suggested that we should find increased acidity as we go north. There is no apparent relation between color and acidity, except that light colored waters usually show slight acidity. The acid nature of the soil solution is a factor in the competition between different species for the occupancy of bog areas.

Bog soils are notably deficient in potassium and available nitrogen. Nitrifying bacteria are prevented from carrying on their normal activities by the acidity of the soil solution, by the lack of oxygen, and by the lower temperature of the substratum.

With few exceptions bog plants are light-demanding forms; hence, in their competition with one another, size and shading ability are prime factors.

That the conditions in the Huron valley are at present not as favorable to the bog plants as to the swamp plants, is shown wherever the two societies come into competition. This fact must be contrasted with the situation in the optimum region of the distribution of bog plants, where the opposite relation has been shown to exist.

An examination of all the physical and chemical data now available fails to account for the differences in flora of bog and swamp areas in this region. The most important factor is believed to be their physiographic history. Where the habitat dates back to Pleistocene times and has remained undisturbed, we find today the bog flora. Where the habitat is of recent origin or has been recently dis-

turbed, we find the swamp flora, or mixtures of swamp and bog species.

The nature of the bog plant societies of the Huron basin is shown by the description of several local bogs, selected to show both the local bog flora and the variation in societies, and arranged to present the genetic changes in a bog flora as a basin filled by peat accumulation. It is shown that during the early stages of bog development, bog sedge, bog shrub, and conifer societies follow each other in the invasion of the basin. These several societies may vary considerably in composition, but they are closely related and show every gradation in a definite order of succession. The bog conifers, however, show no relationship to the surrounding broad-leaved forests of the upland. On the other hand, where clearing has occurred, swamp sedges, swamp shrubs, and swamp trees gain the ascendency, and these not only show an order of succession among themselves, but are genetically related to the broad-leaved trees of the region. The bog societies are part of the northeastern conifer forest formation, while the swamp societies are related to the southeastern broad-leaved forests.

An anatomical study of the bog plants shows that epidermal and hypodermal tissues are thick-walled, that a heavy cuticle is present, frequently supplemented by wax and hairs. Resinous bodies are to be found in the roots and leaves of many of the plants. The leaves are usually small and revolute-margined. Palisade tissue makes up a large part of the mesophyll. Mycorhizas are present in most of the plants. Bog plants resemble the plants of dry sand plains in reduction of foliage area, in development of protective coverings for above-ground parts, and in palisade tissues, but differ from the latter in the matter of root development and root structures.

Experiments indicate that the local bog water itself has no tendency toward the production of xerophilous modifications. Low soil temperatures and lack of soil aeration, however, cause a reduction in the development of the several plant organs. When these two factors are combined, the effect is very marked.

Experiments with *Rumex acetosella* are of especial interest in that nearly all of the characteristics of bog plants may be developed either by lowering the soil temperature, as compared with the air temperature, by preventing proper soil aeration, or by growing in

dry sand. Palisade tissue was developed in the leaves of these plants in diffuse light, and it is shown that palisade tissue is to be correlated with physiological drought. An analogy between the bog habitat and the dry sand habitat is established.

Experiments with Larix indicate that mycorhizas develop only in poorly aerated substrata; their growth is exceedingly slow; the acidity of the substratum is not a factor in their development; a naturally or artificially aerated substratum favors the development of normal roots, and these roots when not surrounded by water develop root hairs abundantly. Larix seedlings can withstand prolonged submergence. When exposed to low substratum temperatures and poorly aerated soil conditions, Larix produces more xerophilous leaves.

Further field work on the bog plant societies needs to be carried on in the region extending from Winnipeg to New Brunswick. Data on the soil and air temperatures, the acidity, the chemical composition of the soil solution, and the plants associated in bog areas throughout this region will go far toward solving the problems of the distribution of bog plants. Experimentation on the production of xerophilous structures by bog conditions should be continued on a larger scale than is possible in the ordinary university plant-house.

To Professor V. M. SPALDING and Professor F. C. NEWCOMBE, of the University of Michigan, under whose direction this work was planned and carried out, I desire to express my sincere thanks both for helpful suggestions and the facilities of the institution which were freely placed at my disposal. Many thanks are also due Professor I. C. RUSSELL for criticism of the physiographic part of this paper. I wish to acknowledge the kindness of Mr. FRANK LEVERETT, of the U. S. Geological Survey, whose intimate knowledge of the glacial geology of this region has been most helpful to me in the prosecution of my own field work. To Mrs. N. L. BRITTON I am indebted for the determination of the mosses. Finally I take this opportunity to express my appreciation of my friend and former instructor, Dr. H. C. COWLES, to whose writings and lectures I owe my interest in ecological botany.

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NUCLEAR DIVISION IN ZYGNEMA.

MABEL L. MERRIMAN.

(WITH PLATES III AND IV)

THE species of Zygnema chosen for this investigation possesses a nucleus unobscured by chromatophores, and hence one in which division stages can be easily followed. No zygospores were found in the material, so the species could not be identified with any degree of accuracy. The number of pyrenoids are normally two, one on each side of the nucleus. The material was gathered from the same locality, the margin of a brook, during the months of August and September of two successive years. The filaments were studied in a living condition to make sure of the presence of dividing nuclei, and were then killed in chromacetic acid and the weaker solution of Flemming for later study. The greater part of them were killed in the evening, as it was also desired to secure division stages of other Conjugatae, which grew in great abundance in the locality and have been reported by investigators as dividing more actively at night. Of these, three species of Spirogyra and two of Mesocarpus will furnish the material for a later contribution.

As nearly all the literature upon the cytology of the Conjugatae relates to forms of Spirogyra, its consideration will be deferred until the completion of further studies in the nuclear division of the group. It is hoped then to bring into accord all the observations as to the character of chromatin and nucleoli.

Filaments of Zygnema treated with the combination stain of safranin and gentian violet, were found upon examination to have retained the violet only in the cell sheath, while the nuclear structures and pyrenoids retained the safranin. Various results were obtained with those treated with Heidenhain's haematoxylin in combination with iron alum and eosin. As the same length of exposure to the stain did not suffice for Spirogyra and Mesocarpus growing entangled with the Zygnema, the material was allowed to remain in the staining fluids for a shorter or longer time. Filaments show pyrenoids stained black by the haematoxylin, the nuclear structures retaining the eosin; or the pyrenoids may be stained red by the eosin, and the

nuclear structures black by the haematoxylin; or finally both may appear stained red by eosin. Such differences are shown in the drawings from the different preparations; the parts shaded in black represent portions stained by the haematoxylin, as in *fig. 33*, those in gray the portions stained by the eosin, as in *fig. 13*.

Within a quiescent nucleus situated between the two pyrenoids thus stained, there can be seen a central body stained somewhat redder or blacker, as the case may be, than the peripheral network of granules. This network of granules, ordinarily scarcely distinguishable from the cytoplasmic reticulum, was found in some cases to be quite conspicuous.

If an examination is made of a nucleus in process of reconstruction from the telophase, within the forming membrane can be seen a conglomerate mass of substance, very evidently non-homogeneous both in surface view and as seen in outline, *figs. 1, 39*. Around this smaller bodies can be seen in the meshes of a delicate network. The staining capacity of the larger mass and the small bodies varies in the different preparations; in some instances they are sharply defined from one another, at other times they retain the same kind and amount of stain.

It cannot be denied, however, after a careful examination of stages preceding the appearance of these bodies, that the substances in both came from the chromosomes of the metaphase. Bearing in mind, then, that the large mass and the smaller granules have the same origin, it would hardly seem correct to discriminate between the two, terming the one nucleolus and the others chromatin granules. Neither method of staining nor study of their history yields evidence other than that they are of similar substance, differing only in position and aggregation. It is as if in the revolutions going on within the cell some of the chromatin granules had been drawn to the center, there incompletely cohering, while others were left at the periphery. In describing, then, the quiescent nucleus of Zygnema it seems preferable to say that the larger portion of the chromatin granules cohere to form a central body analogous in its position to the nucleolus of higher plants.

The division of the nucleus is presaged by granules collecting in the region where the cell wall will form. The activity of these vibrat-

ing granules in the living cell renders the nuclei about to divide easily distinguishable from the remainder in the filaments. Owing to the activity of these granules, changes going on within the living nucleus could not be easily followed, but changes in the form and position of the nucleus together with those of the pyrenoids were followed throughout division. Accordingly the history of changes in the chromatin is all deduced from comparison of dividing nuclei stained by haematoxylin or safranin as outlined above.

If haematoxylin in combination with iron alum could be considered as an infallible criterion for distinguishing chromatic from achromatic material, and stages could be selected from material stained by one of the methods only, it would be an easy matter to trace the history of this central body originating from the chromosomes of the metaphase. Often, as in *fig. 2b*, numerous deeply stained bodies are to be seen lying in the space surrounded by a membrane, with no trace of chromatin bodies without. In the nucleus represented in *fig. 5*, in place of the central body several smaller bodies can be seen marked off from the eosin-stained bodies by the blackness of the stain. Passing to *fig. 9*, where the beginnings of an intranuclear spindle are manifest, and where there are several more deeply stained bodies, and then to *fig. 12*, where six discrete bodies distinctly form an equatorial plate, the natural conclusion, based wholly upon similar staining properties, would be that the central mass of chromatin alone furnishes the chromosomes for the equatorial plate. Such was the conclusion reached during the first year of this investigation, but further study of the material shows it to have been premature, or, if applicable at all, only to a few cases. The conviction that difference in staining of nuclear structures is more often a matter of manipulation than of chemical reaction, and that difference in the shade produced by the stain is merely due to the density of the body and time given for penetration, renders necessary in interpretation a great degree of caution.

The following account is derived from a comparison of parallel stages in all the preparations.

As the nuclei pass from the quiescent to the active state, the centrally lying mass disintegrates into small bodies (*figs. 2, 3*); at the same time the granules lying at the periphery increase in size. The

space within the nucleus becomes gradually clearer (*fig. 5*), the nuclear sap probably reinforcing the substance of the granules. As the result of the disintegration of the central body and the growth of the other granules, there may be seen lying within the nucleus twenty or more granules (*figs. 4, 5, 6*). In a few cases these bodies may slightly cohere, but in the majority of cases they lie free. No cases were found here or in later stages of the formation of a spirem. In many instances all the bodies within the nucleus retained merely the eosin stain (*fig. 6*), and hence were entirely undifferentiated from each other. In a few cases, like *figs. 5, 7, 8*, some of the bodies retained only the black stain from the hæmatoxylin. In one instance (*fig. 11*), a faintly stained larger body, with one or two smaller ones of similar shade, can be seen lying within the nuclear space, surrounded by numerous more deeply stained granules. If the other stages mentioned had not been observed, the latter faintly stained body might have been interpreted as a nucleolus like those in higher plants, now in the act of becoming dissolved in the cytoplasm. Extended comparison, however, of parallel stages justifies the view that this body is only a portion of the central mass of the quiescent nucleus, about to undergo still further disintegration into chromosomes.

The many chromosomes thus resulting approach one another (*figs. 6, 9*), presenting in many cases an appearance analogous to the synapsis stage described as occurring in the higher plants. Finally they become arranged in a circle concentric with the short axis of the cell. In one case (*fig. 10*), such an arrangement was observed before the nuclear membrane became dissolved. *Fig. 14* shows this massing of granules in the equatorial plane after the dissolution of the nuclear membrane. The chromosomes in this cell were all stained black, but some were drawn in lighter tint to show that they were lying in three different planes. *Fig. 15* also represents a similar stage, and *fig. 18* one somewhat further advanced. The chromosomes now appear to be denser than in previous stages, an interpretation based upon the circumstance that the hæmatoxylin stain does not as readily become washed out.

After having formed the ring they appear to be drawn inward, becoming denser and undergoing a process of fusion. By this draw-

ing-in process they come to lie in two closely adjoining parallel rows. As no case of a single row of isolated granules in the same plane was found, there is no evidence that such double row was produced by the division of a single row. *Fig. 25* represents two rows of chromosomes lying in the same plane. In *fig. 12* fusion has taken place to such an extent that only three chromosomes are present in each row.

Many of the chromosomes presented a tetrahedral appearance, as in *figs. 16* and *20*, thus pointing to the conclusion that the fusion of the condensing granules may take place in fours. In some cases the fusion has gone so far as to result in only four groups of tetrads (*figs. 22, 23, 21*). *Fig. 24*, of a more highly magnified group, shows especially distinctly this grouping of the granules. Careful focusing on this stage indicated the presence of another underlying group. As many cases were found of such grouping of the chromosomes in fours, it does not seem that it could have been purely accidental.

When the maximum amount of fusion and condensation is reached, the limit apparently varying in different cells, each half of a group becomes dissociated from its adjoining members and gradually draws away, as in *fig. 27*. In process of separation each group becomes broken up into smaller groups, in the meantime all becoming again arranged in two rings concentric with the short axis of the cell (*fig. 26*).

Thus numerous chromosomes are arranged in a circle in stages preceding and immediately following the stage of the equatorial plate, in which commonly four to six chromosome groups may be seen. It seems difficult to believe that six chromosomes (*fig. 12*) could have resulted directly from condensation and fusion of thirty or more chromosomes (*fig. 14*). A comparison of chromosomes as to size and staining qualities in the two adjoining cells (*figs. 12, 13*), drawn with Abbé camera, would certainly indicate that each chromosome must suffer a loss of its more liquid substance in the process of being drawn into the equatorial plate, or that a few must be entirely dissolved. Whether all condense to form a few, or whether only a few are chosen to transmit the chromatin to daughter nuclei, the remainder becoming dissolved in the cytoplasm, cannot be stated with certainty, as the staining process does not solve the problem as to the fate of the individual granules. When *all* the preparations are

examined and not a selected few, there seems to be more evidence of the first being the true account of events.

It was thought at first that this difference in number of chromosomes might be due to difference of species, as none of the Zygnema examined had zygospores, and hence it is possible that two or more species might have been growing together. The discovery of cells like those in *figs. 6, 12, 13, 15, 23, 22*, in the same filament is indisputable evidence that in the same individuals the number of chromosomes decreases from thirty or more down to six or eight, and then increases to thirty or more. This change in number occurs in a few moments, as determined in living cells by the changes in the position of the nucleus. All the filaments were examined in surface view, so it cannot be maintained that the number of chromosomes had been increased by sectioning.

As the rings of chromosomes approach the chromatophores, the cytoplasm is condensed on the side nearest the chromatophore. The explanation of this might be that a large part of the cytoplasm which is not diverted to the region of the formation of the cell plate was streaming in toward the center, as in *figs. 14–18*, while in *figs. 26–30* it was streaming out towards the chromatophores; that the chromosomes are forced together by the inflowing streams and in the vortex of opposing currents become dissociated. The word "dissociated" is used in preference to the word "splitting," as there appears to be no evidence of splitting and hence of equal distribution of homogeneous bodies. The chromosomes being heavier than the cytoplasm, the condensation appears on the side nearest the chromatophore (*figs. 28, 29*).

It is to be regretted that in the living cells chromosomes could not be distinguished from actively vibrating granules in the cytoplasm. Nothing could be discovered which in any way resembled spindle fibers, although streams of granules and the alternating space of nuclear activity was easily traced.

The number of chromosomes finally arriving at the chromatophore may be fifteen to twenty in each ring, as in *fig. 30*. The cytoplasm, being somewhat arrested in its flow by the chromatophore, causes a change in the position of the chromosomes. The majority, as they undergo still further dissociation, are drawn to the center, incom-

pletely cohering, while a few appear lying in delicate strands about them (*fig. 31*). In some cases all the chromosomes may cohere to form the central body. The nuclear membrane now emerges from the condensation of cytoplasm (*fig. 32*). As the chromosomes are now shut off from the influence of currents in the cytoplasm they generally remain unchanged in position, fusing either to form one mass (*fig. 33*), or three or more smaller masses (*fig. 34*), or rarely (*fig. 35*) all the chromatic material may be diffused in the nuclear plasm, forming numerous more or less tetrahedral granules.

It is to be noted that not until the chromatic rings have separated and have approached the chromatophores do the pyrenoids ordinarily show any evidence of division. This observation was easily confirmed from the study of living cells. *Fig. 29* represents the only one seen, out of many filaments examined, in which the pyrenoids divided before the formation of the nuclear membrane. As the newly divided nuclei approach their respective chromatophores, one or both plastids begins to show a constriction. This deepens until when the nuclei come to lie directly over, only a narrow band of less dense substance resembling linin connects the two daughter pyrenoids (*figs. 37, 38*). This becomes gradually reduced until it appears only as a thread (*fig. 39*). Later the nucleus sinks down and the separation is complete. The constriction of the plastids forming the center of the two pyrenoids takes place synchronously, as is the case with the stages in the daughter nuclei. One instance only was observed in which one plastid suffered division when other plastids had just begun to elongate (*fig. 32*).

Although division of the pyrenoid may be influenced by division of the nucleus, that it is not wholly dependent upon it was demonstrated by leaving actively dividing filaments of Zygnema for one hour in a watch crystal containing 10^{cc} of water to which two drops of chloroform were added. There were but few visible signs of plasmolysis in filaments killed and stained as in other material, but while a majority of the nuclei had ceased to divide, a majority of the pyrenoids were dividing as in normal filaments. That this division was not merely fragmentation was shown by sequence of stages and the presence of the band connecting the plastids. Fragmentation of the pyrenoids took place in filaments in stronger solutions of water

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and chloroform in which plasmolysis occurred to a much greater extent.

Hence, cytoplasmic streams, nuclear structures, chromatophores, and pyrenoids take an active part in the division of cells in Zygnema. The streams of granules, collecting where the cell plate is to form, marks the beginning; the nuclear changes then proceed, followed by division in chromatophores and pyrenoids, while all are correlative with the formation of the cell plate.

It cannot then be said that division of the nucleus, the chromatophores, and the pyrenoids are synchronous. Rather is it true that the center of activities of the cell shifts, and with this shifting division of the bodies lying in the vicinity occurs. As regards the nuclear structures in Zygnema it is apparent that there are no bodies analogous to the nucleoli found in the higher plants. A large portion of the chromatin, or in a few cases possibly all, fuses in the anaphase to form one or more bodies corresponding in appearance and position to that of nucleoli of higher plants. Instead of waste products of chromatin condensing to form one or more bodies in the nucleus, the waste products are not separated from the chromosomes, but retained in them until after the nuclear membrane disappears in the next division. The substances which make up chromosomes and nuclear waste products, if such we may rightly regard the nucleoli of higher plants to be, are in Zygnema morphologically indistinguishable.

The history of chromatin before the formation of the equatorial plate may be summarized as consisting of growth, association, and condensation of chromatin bodies in groups. These groups may be partially coherent, but in no case form a spireme. After equatorial plate formation, dissociation into groups follows, continuing until the chromosomes reach the chromatophores.

Although the term chromosome has been used in this account, researches as yet incomplete make it exceedingly doubtful whether the chromatin bodies in any of the Conjugatae are to be regarded as at all homologous with chromosomes of higher plants. If we restrict the term chromosomes to segments of the tubular spirem,[1]

[1] See MERRIMAN, Vegetative cell division in Allium, BOT. GAZETTE **37**:178-207. *pls. 11-13*. March 1904.

then the chromatin bodies seen in *figs. 14* and *15* of Zygnema cells are homologous not with the chromosomes of Allium but with the granules seen in the earliest stage of the spirem, while groups in *figs. 16* to *23* are directly comparable with the groups or rings of tetrads, which in Allium fused to form the tubular chromosomes.

Zygnema possesses a mechanism of nuclear division less elaborated than that of the higher plants, inasmuch as dissociation of chromatin bodies occurs immediately after their association into primary groups without the intervention of a spirem. From this point of view appearances observed in Zygnema support the interpretation suggested in my account of nuclear division in Allium, namely, that the chromosomes are formed by fusion of bodies in groups, and that when a longitudinal splitting appears it is not to be considered a true splitting of a homogeneous substance but rather a dissociation of bodies which from the first were discrete.

If this be true, then doubts may reasonably be entertained as to the validity of the conception held by ROUX, and successively by many other investigators, that the complex apparatus for indirect division of the nucleus exists for the purpose of enabling each chromatin body to furnish its quota to the daughter nuclei.

The essential feature of indirect division, and therein its advantage over direct division, appears to be the dissolution of the nuclear membrane. Thus is made possible a free interchange of nuclear and cytoplasmic substances and a renewal of the vitality of the cell.

Zygnema, then, may be considered as furnishing additional evidence of interchangeability of nucleoli and chromatin bodies, of variability in their number, and negatively as furnishing no evidence that equal distribution of chromatin is effected by either transverse or longitudinal splitting of homogeneous bodies. Nuclear structures, cytoplasm, pyrenoids, and chromatophores are transferred in equal amounts to the daughter nuclei and by a process differing not fundamentally in the result from that which would have been attained by direct division.

NORTHFIELD, MASS.

EXPLANATION OF PLATES III AND IV.

The figures were drawn with the aid of an Abbé camera.

PLATE III.

FIG. 1. Daughter nucleus from a cell where the cell plate is not yet completed. The nuclear structures in this cell retained the eosin stain, the pyrenoids black from haematoxylin. × 1750.

FIG. *2a*. Nucleus preparing to divide, showing growth of bodies in the peripheral network before breaking up of the central body. Pyrenoids and nuclear structures in this cell retained only the eosin stain. × 1750.

FIG. *2b*. Nuclear material stained black by the haematoxylin, all the chromatic material being apparently condensed in the space occupied by the central body. × 1750.

FIG. 3. Nucleus showing the breaking up of chromatin body and increase in size of the peripheral bodies. The pyrenoids retained the eosin stain; all the nuclear structures are stained black, several of them somewhat darker than the others. × 1750.

FIG. 4. Nucleus showing the beginning of the massing of the chromosomes, the nuclear membrane as yet undissolved, the granules in the region of the cell plate formation being conspicuous. Chromosomes black, pyrenoids red.

FIG. 5. Later stage, showing the clearing of the nuclear interior, recalling the synapsis stage described in higher plants. Pyrenoids red, several chromosomes black, remainder red. × 1750.

FIG. 6. Similar stage, very frequent; chromosomes numerous, massed together, all stained red. × 1750.

FIGS. 7, 8. Similar stages where there is no massing of the chromosomes. In 7, six chromosomes were stained black, others red. In 8 those stained black are grouped in one corner of the nucleus, those red are scattered. × 1750.

FIG 9. A stage where distinct lines of granules connect chromosomes with nuclear membrane. Four chromosomes black, others red. × 1750.

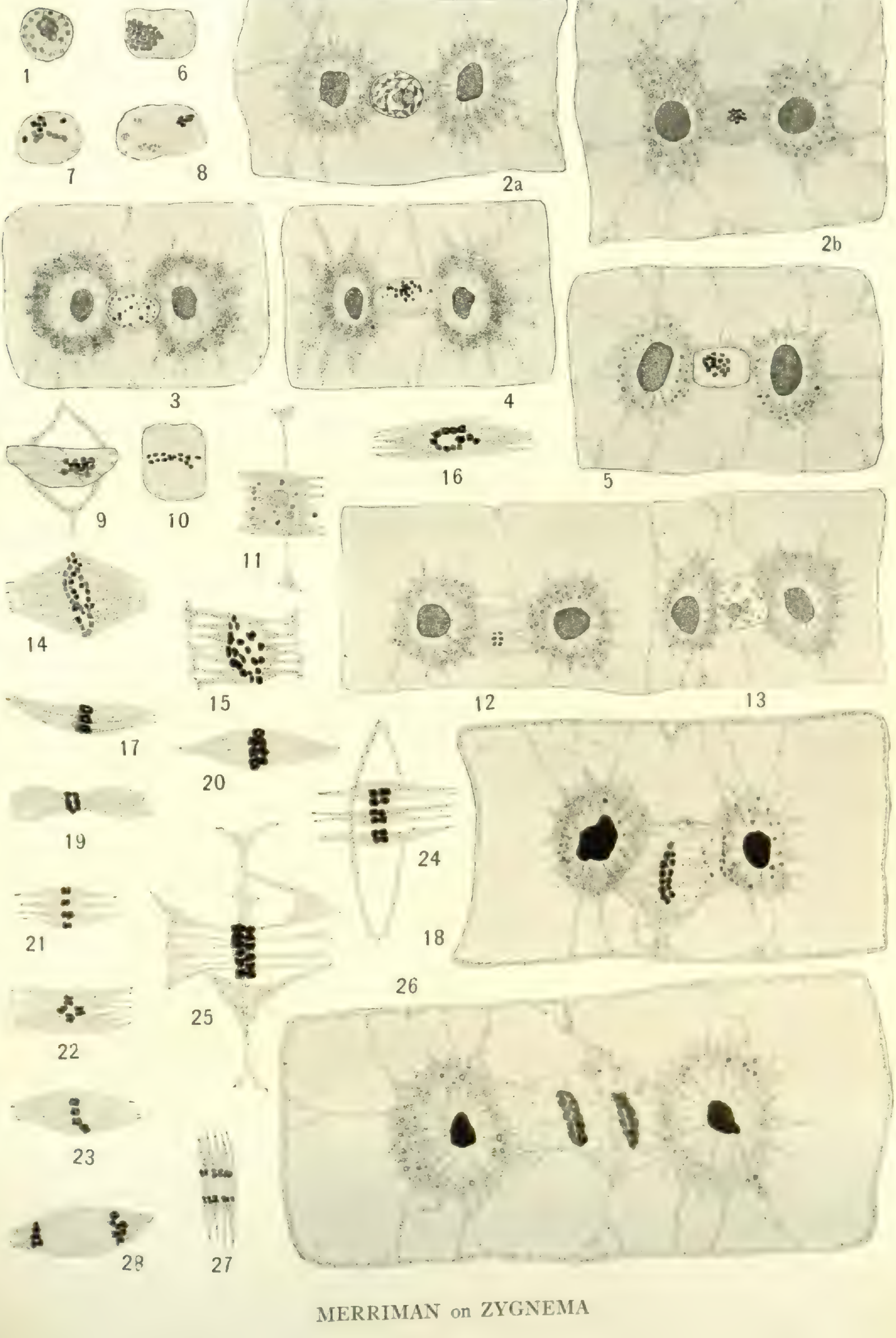
FIG. 10. A rare stage with numerous chromosomes arranged in circle within the nucleus before the nuclear membrane becomes dissolved. All chromosomes black. × 1750.

FIG. 11. Another rare stage; nuclear membrane dissolved, remains of central body still in the cytoplasm, retaining a lighter eosin stain than the other chromosomes. × 1750.

FIGS. 12, 13. Two adjoining cells in same filament showing disparity in size and number of chromosomes. Pyrenoids red, in *fig. 12* chromosomes stained sharply by haematoxylin; in *fig. 13* nuclear structures stained red. The line of granules marking the region of cell plate formation shown in both figures. × 1750.

FIG. 14. All chromosomes black, but some drawn lighter to indicate that they were lying in three different planes. × 2440.

FIG. 15. Chromosomes black, showing indefinite arrangement as they are being drawn to the center. × 2440.



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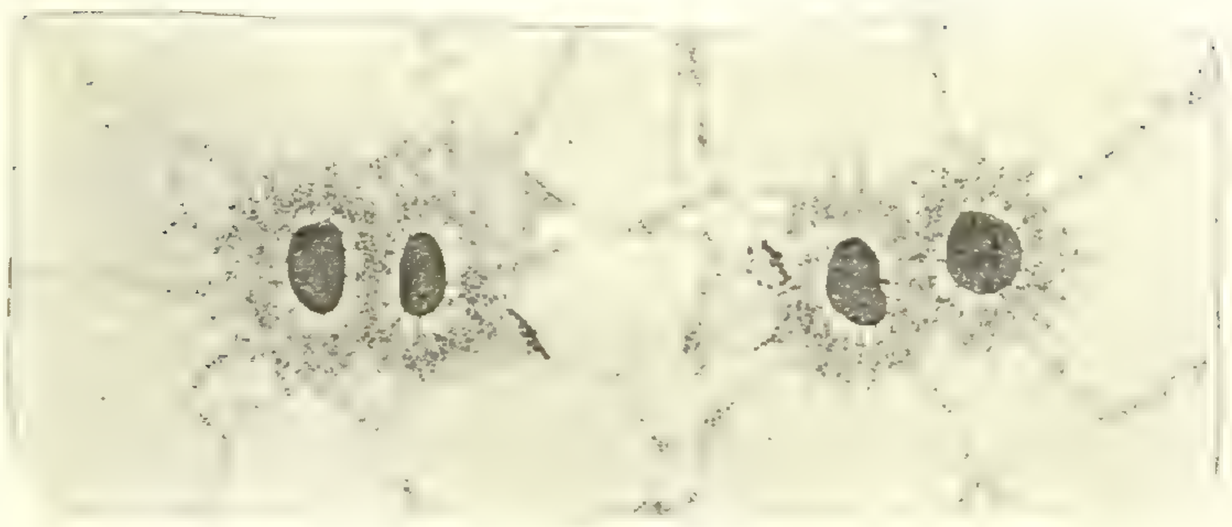

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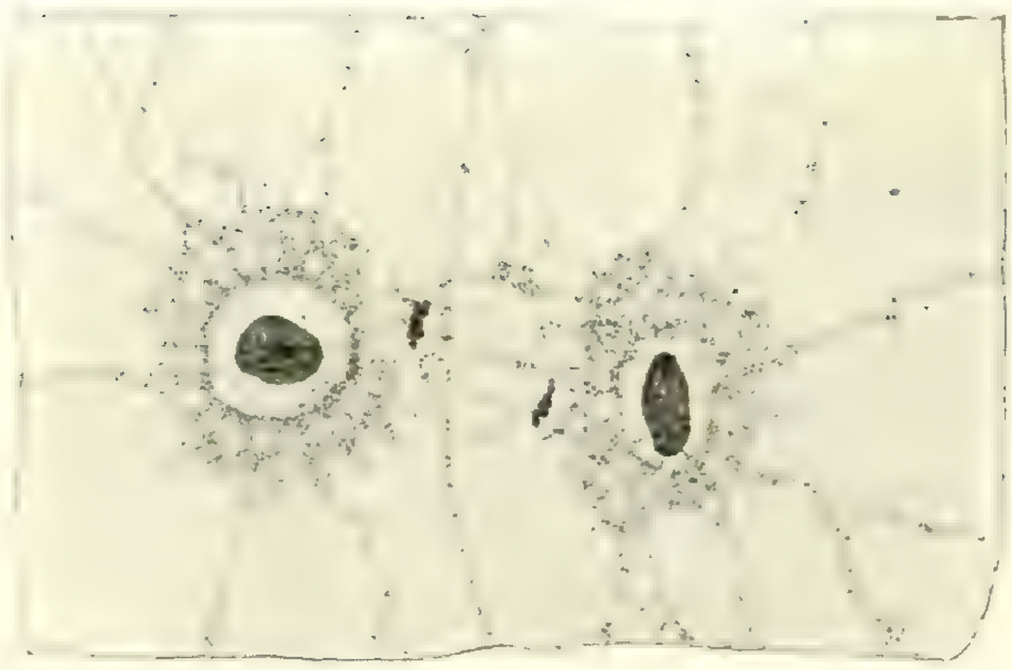

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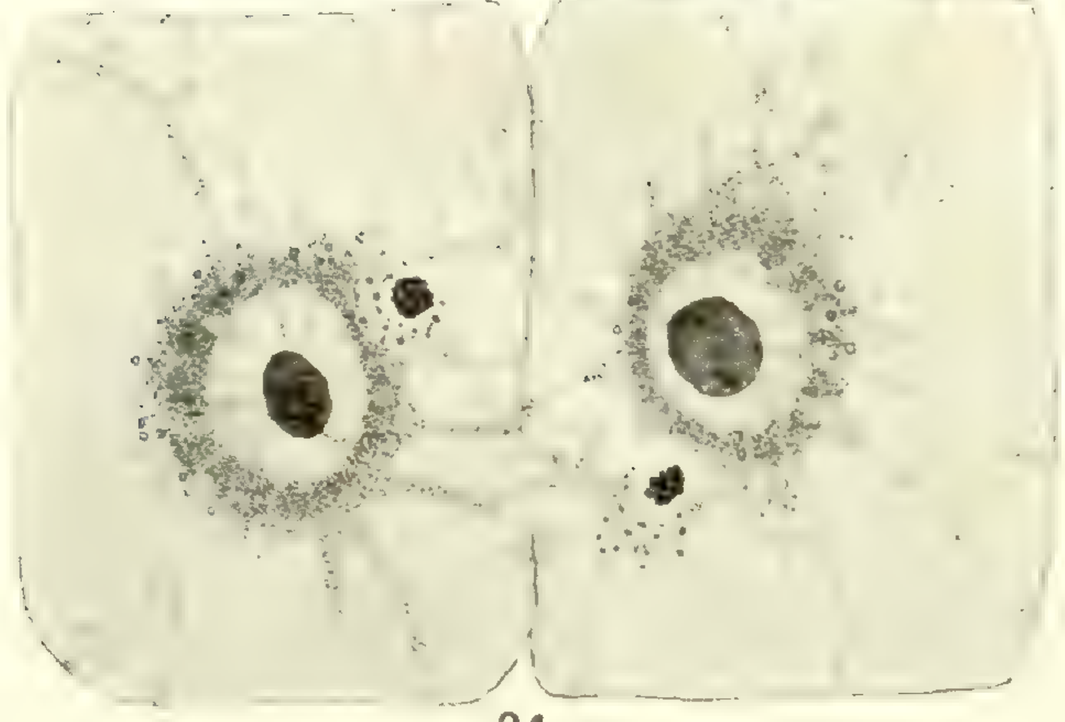

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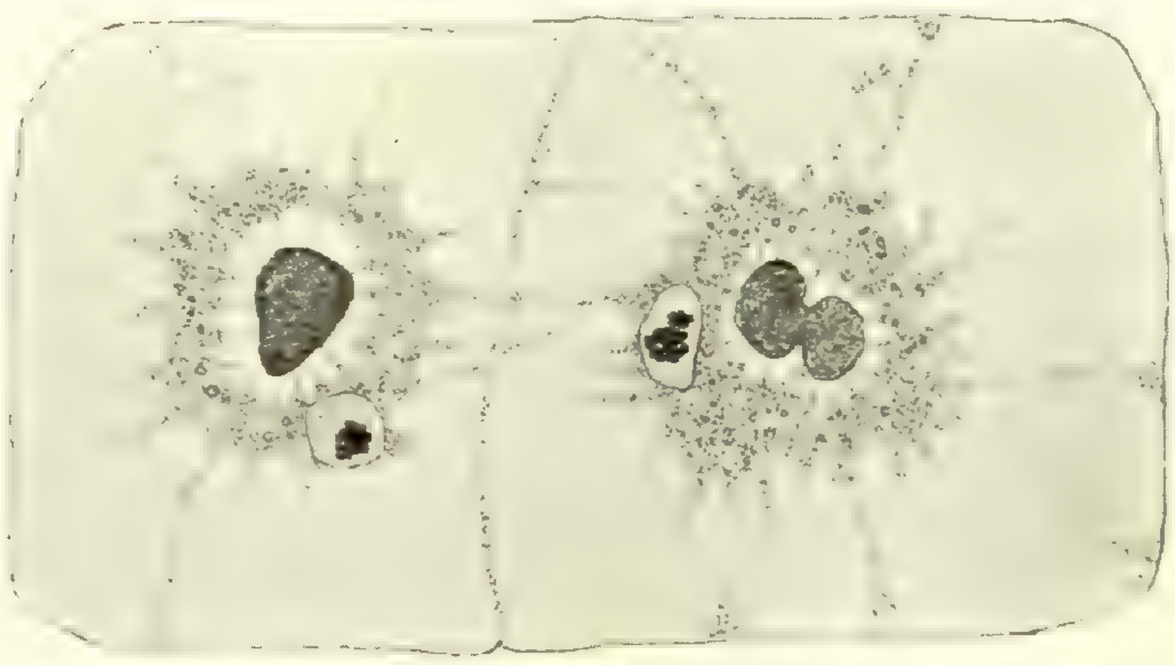

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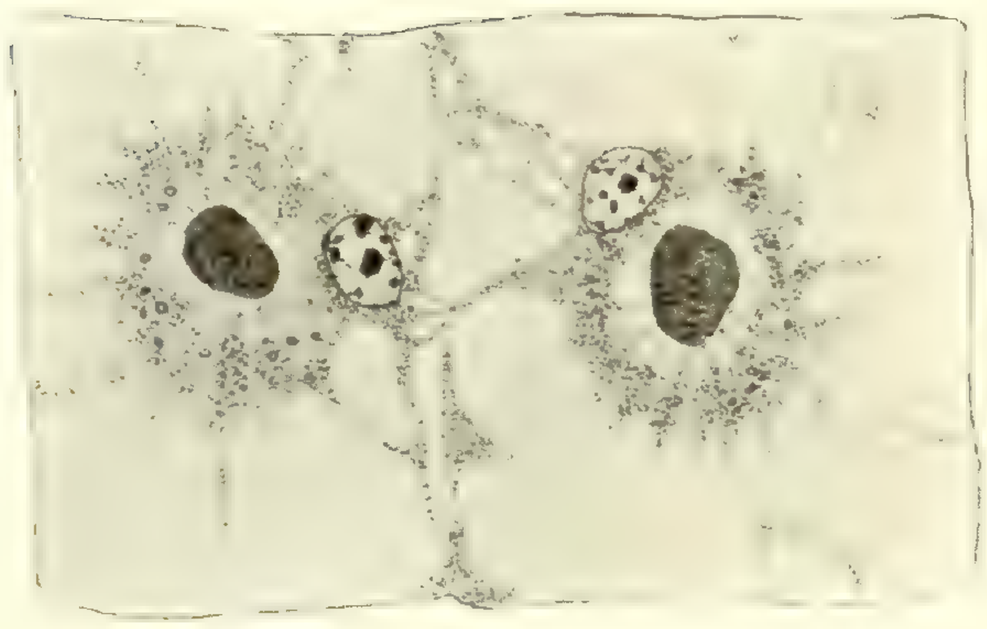

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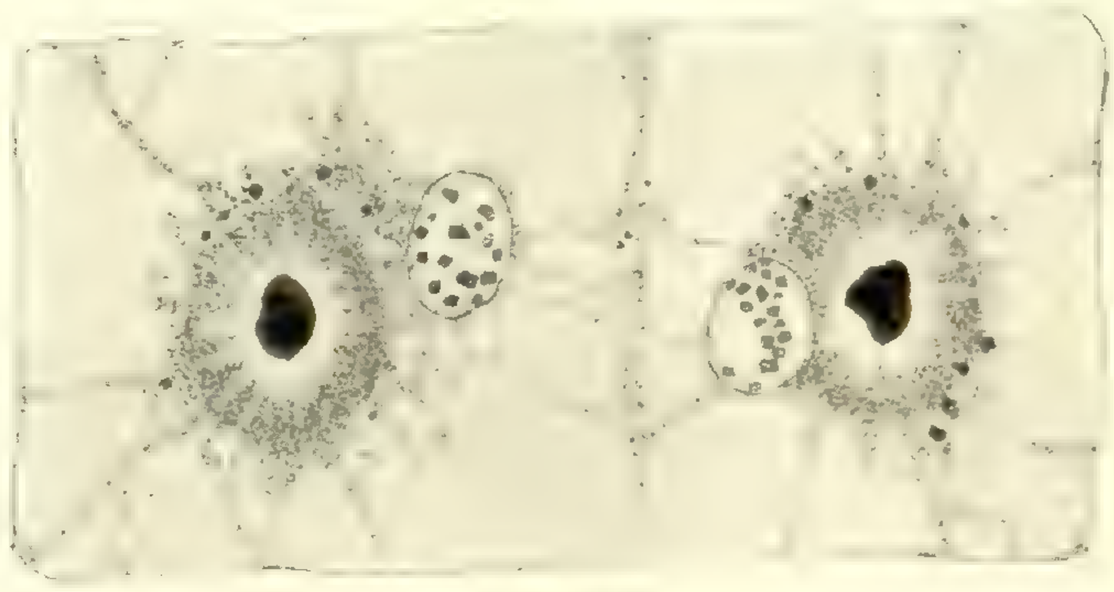

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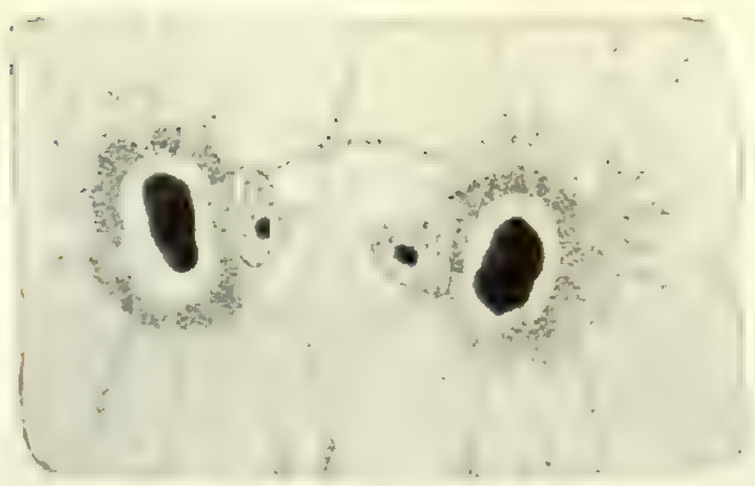

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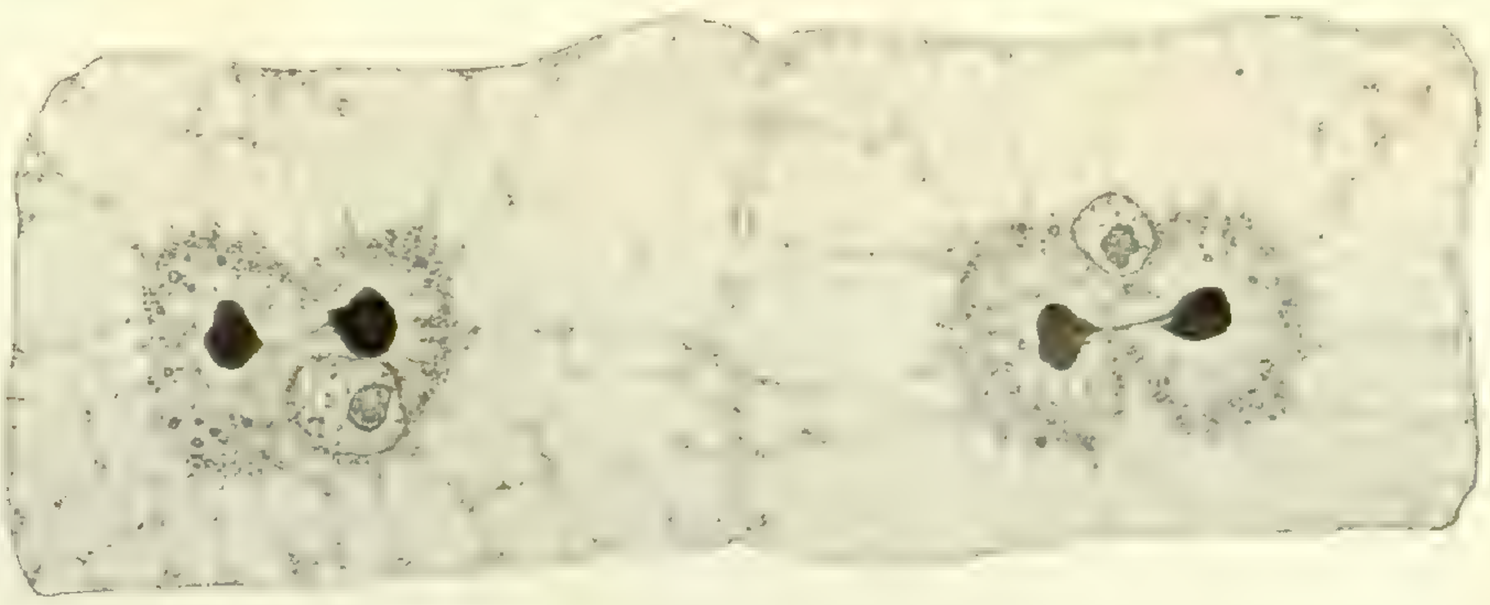

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FIGS. 16, 17, 19, 20, 21, 22, 23 show successive stages in condensation of chromosomes. Chromosomes all black. × 1750.

FIGS. 18, 24, 25, 26. Chromosomes and pyrenoids black. × 2440.

FIGS. 27, 28. Chromosomes becoming dissociated into smaller groups. Chromosomes black. × 1750.

PLATE IV.

FIG. 29. An unusual case of division of pyrenoids before formation of membranes of daughter nuclei. Chromosomes black, both in central mass and in the periphery, pyrenoids red. ×1750.

FIGS. 30, 31, 32. Chromosomes black, pyrenoids red. ×1750.

FIGS. 33. Pyrenoids and central body of nucleus black, peripheral bodies red. ×1750.

FIG. 34. Pyrenoids red, all the nuclear bodies black. ×1750.

FIG. 35. Nuclear bodies red, pyrenoids black. ×1750.

FIGS. 36, 37, 38. All nuclear bodies red, pyrenoids black. ×1750.

FIG. 39. Nuclear bodies red, pyrenoids black, showing vestiges of connecting substance. × 1750.

EFFECT OF CERTAIN SOLIDS UPON THE GROWTH OF SEEDLINGS IN WATER CULTURES.[1]

J. F. Breazeale.

(WITH FOUR FIGURES)

In certain investigations on the growth of wheat seedlings in aqueous extracts of soil, it was observed that the growth of these plants was greatly accelerated by the presence in the medium of undissolved calcium carbonate. That the observed acceleration was not due to an increase in dissolved calcium was apparent from the fact that the presence of other slightly soluble salts of this element failed to produce any response. It appeared possible that the effect of calcium carbonate might be due to its taking up some injurious substance present in the extract. This was suggested by Nägeli's well-known discovery[2] that water, which is toxic to algae because of minute traces of metals, can be improved by placing in it such insoluble bodies as graphite, paraffin shavings, or torn filter paper. It was determined to try other slightly soluble compounds which might remove from solution small amounts of solutes, either by chemical action or mechanically. The results of this investigation make up the present paper.

The Russian variety of wheat known as "Chul," obtained from Arizona, was used in most of these experiments. The seedlings were germinated in sand and then grown in water cultures in large-mouthed black bottles of about 60^{cc} capacity. They were fixed in cork stoppers, four in a bottle, in the manner described by Whitney and Cameron[3] for cultures of this kind, so that the roots were submerged in the solution while the seeds were just above its surface. The solutions were always aerated by violent and repeated shaking before the cultures were started. During the growth of the plants the bottles were weighed in groups of three at intervals of three or four days, and the water lost was replaced with distilled water. The manner

[1] Published by permission of the Secretary of Agriculture.

[2] Nägeli, C. von, Ueber oligodynamische Erscheinungen in lebenden Zellen. Denkschr. Schweiz. Naturforsch. Gesell. **33**: 1. 1893.

[3] Whitney, M., and Cameron, F. K., Investigations in soil fertility. U. S. Dept. Agric., Bureau of Soils, Bull. 23. 1904.

of fixing the seedlings practically prevents water loss, except through the transpiration of the plants. The work of LIVINGSTON[4] indicates that total loss by transpiration for a period of two or three weeks furnishes a fair criterion for comparison of the growth of different cultures of wheat grown in this manner. The transpiration figures are used in this way in the experiments here given. The work was carried on in a greenhouse with a temperature of 15 to 25° C.

For Experiments I to III a soil extract from poor Leonardtown loam, collected near Leonardtown, Md., was used. It was prepared by stirring the soil for three minutes with water in amount equal to twice its air dry weight, allowing it to stand twenty minutes, and then filtering through a clean Pasteur-Chamberland filter tube, in the manner described by WHITNEY and CAMERON.[5]

In Experiment I the solids used were calcium carbonate, tricalcium phosphate, ferric hydrate and aluminum hydrate. Ferric hydrate was prepared by precipitation from the chlorid with ammonia, followed by thorough washing with hot water. It was transferred moist to the culture media. Aluminum hydrate was prepared in a similar way from the sulfate. Data for this and the three following experiments are given in Table I. The percentage increase in transpiration for each solid is computed by considering the transpiration from the untreated extract as unity.

All of the solids accelerated growth, as is shown by the transpiration figures. But in the case of the ferric hydrate the root growth was accelerated to a much greater extent than that of the tops. The roots of the culture with this substance were much longer than those of the other cultures. It is evident here that root growth was accelerated without a corresponding increase in transpiration.

Experiment II comprised, besides calcium carbonate and ferric hydrate, carbon black (prepared from burning petroleum, and thoroughly washed), magnesium carbonate, and barium carbonate. The small amounts of water transpired are due to cloudy weather.

The plants of this series are shown in *fig. 1*, the numbers in the figure corresponding to the culture numbers given in parentheses

4 LIVINGSTON, B. E., Relation of transpiration to growth in wheat. BOT. GAZETTE 40:178–195. 1905.

5 WHITNEY, M., and CAMERON, F. K., The chemistry of the soil as related to crop production. U. S. Dept. Agric., Bureau of Soils, Bull. 22:16 ff. 1903.

in the table. It will be noticed that carbon black shows the same tendency to produce abnormal root growth as does ferric hydrate, but to a less marked degree.

Experiment III included very finely pulverized quartz flour, as well as ferric hydrate and carbon black. The two last named bodies showed here the same abnormal acceleration of root growth as was previously observed, but quartz flour, although it improved the general growth of the plants, produced no such effect.

FIG. 1.—Experiment II; 24 wheat plants grown 19 days. 1, Extract of Leonardtown loam; 2, the same with calcium carbonate; 3, the same with ferric hydrate; 4, the same with carbon black; 5, the same with magnesium carbonate; 6, the same with barium carbonate.

The experiment with ferric hydrate and carbon black has been repeated many times with extract of Leonardtown loam, as well as that of other soils, and always with the same result. In some cases acceleration of root growth is more marked with carbon black than with ferric hydrate, but usually the reverse is true.

Experiment IV was carried out with an aqueous extract, prepared as above, from Miami silt loam collected at the Rhode Island Experiment Station, at Kingston, R. I. This soil had been in hoed crops

for ten years without fertilizer, and was acid to litmus paper. To make absolutely sure that the effect of carbon black was not due to any substance added with it, the distilled water for the soil extract was shaken with the solid carbon black and filtered through a

FIG. 2.—Experiment IV; 36 wheat plants grown 13 days. 1, extract of Miami silt loam; 2, the same with ferric hydrate; 3, the same with carbon black; 4, the same with ferric hydrate, carbon black, and calcium carbonate.

Pasteur-Chamberland tube before being used. Ferric hydrate, carbon black, and a mixture of these two bodies, together with calcium carbonate, were used in this case. The plants are shown in *fig. 2.*

From this experiment and others of similar nature it seems clear

that the effect of the carbon black is not due to any stimulating substance which it carries into the medium. Other experiments have shown that no acceleration of growth is obtained with the addition

TABLE I

DATA FOR EXPERIMENTS I TO IV.

Medium	Experiment I 24 plants grown 19 days		Experiment II 24 plants grown 19 days		Experiment III 12 plants grown 17 days		Experiment IV 36 plants grown 13 days	
	Total transpiration	Per cent. increase	Total transpiration	Per cent. increase	Total transpiration (av. of 2 cultur's)	Per cent. increase	Total transpiration	Per cent. increase
Untreated soil extract.....	154	....	(1) 33	....	51	....	(1) 68	....
Do.+tri-calc. phosphate..	182	18.1	....	....	....	....	....	....
Do.+calcium carbonate..	191	24.0	(2) 81	145.4	....	....	....	....
Do.+ferric hydrate.....	184	19.5	(3)110	233.3	187	266.6	(2) 176	158.8
Do.+aluminum hydrate..	187	21.4	....	....	....	....	....	....
Do.+carbon black	....	....	(4) 69	109.0	154	201.9	(3)139	104.4
Do.+magnes. carbonate..	....	....	(5) 73	121.2	....	....	....	....
Do.+barium carbonate...	....	....	(6) 78	136.3	....	....	....	....
Do.+quartz flour.....	....	....	....	....	87	70.5	....	....
Do.+ferric hydrate, carbon black, and calcium carbonate.....	....	....	....	....	....	....	(4)221	225.0

of iron salts to the extract of poor Leonardtown loam. Therefore, a slight increase in dissolved iron cannot be the cause of the acceleration noted in the case of this hydrate. It seemed probable that ferric hydrate and carbon black had their effect through an active removal, perhaps by mere adsorption, of some injurious substance occurring in the culture medium. Such a substance might have been in the soil extract originally, or might be produced by the plant roots, or both suppositions might be true together. The third alternative proved to be the correct one.

To obtain evidence in this regard, Experiment V was carried out. Four different soil extracts were shaken with carbon black, filtered, and then used as culture media, comparison being made with controls in untreated extracts. The four soils were of two types, a good and a poor soil of the Cecil clay type, and a good and a poor of the Leonardtown loam type, the former from Statesville, N. C., and the latter from Leonardtown, Md. Chemical analysis both of the aqueous extracts and of the solution obtained by digestion with hydrochloric

acid, fails to show any material difference between the good and the poor varieties of these soils, although they are agriculturally quite different. These cultures were grown under the direction of Mr. F. D. GARDNER, in charge of the Division of Soil Management, of this Bureau. They consisted of forty-eight plants and were grown for

FIG. 3.—Experiment V; 48 wheat plants grown 15 days. 1, extract of Cecil clay, good; 2, the same, filtered from carbon black; 3, extract of Cecil clay, poor; 4, the same, filtered from carbon black.

fifteen days. The results are given in Table II; percentage increases are given for each treated extract compared with the same extract untreated considered as unity.

It will be seen from this table that the extract of good Cecil clay

TABLE II.

DATA FOR EXPERIMENT V.

Culture no.	Medium	Total transpiration in grams	Per cent. increase
1	Extract of Cecil clay, good	485	.....
2	Do., carbon filtered	471	−2.9
3	Extract of Cecil clay, poor	407	.....
4	Do., carbon filtered	477	+17.2
5	Extract of Leonardtown loam, good	359	.....
6	Do., carbon filtered	407	+13.4
7	Extract of Leonardtown loam, poor	271	.....
8	Do., carbon filtered	349	+28.7

was not improved by treatment with carbon where the latter is filtered out. All the others were improved, although the improvement in the case of good Leonardtown loam was not as marked as that in case of the poor. The leaf development and general appearance of the plants were essentially in proportion to their transpiration. The roots showed the same acceleration with carbon treatment and filtering that had been observed in experiments in which the solid was left in the solution. *Fig. 3* shows the roots of cultures nos. 1, 2, 3 and 4. From this experiment and from similar ones it appears that a part at least of the injurious matter which is removed by carbon black is in the soil extract at the start.

FIG. 4.—Twelve wheat plants grown 15 days. 1, redistilled water; 2, the same, with ferric hydrate.

A number of experiments were carried out, using water twice redistilled, first from potassium dichromate and sulfuric acid, then from alkaline potassium permanganate, both in glass boilers, con-

densation being carried on in a platinum tube. Upon this water the effects of carbon black and ferric hydrate were tested, the solids remaining in the water during the growth of the plants. The former gives little or no increase in transpiration, the latter a moderate increase, but both solids produce a marked acceleration in root growth. Twelve seedlings grown fifteen days in redistilled water with and without ferric hydrate are shown in *fig. 4*. No. 1 shows those from the untreated water, no. 2 those with the solid. The water here used probably did not contain injurious substances, and therefore the effect of the solids is most probably due to the removal of some injurious exudation arising from the plants. Further, distilled water in which plants have been grown for a number of days is found to give less growth upon replanting than does unused water, and the injurious effect of the used water is corrected by shaking with carbon black or ferric hydrate and filtering off the solid. Thus it seems that wheat seedlings do give off bodies from their roots which are toxic to themselves.

When the work so far recorded was practically completed, the appearance of TRUE and OGLEVEE'S paper[6] on somewhat similar experiments made it seem advisable to withhold publication until some further tests suggested by that paper could be made. These authors find that by the introduction of sand, filter paper, paraffin, or potato starch into solutions of copper sulfate in which seedlings of *Lupinus albus* are growing, the toxicity of the solute is remarkably decreased. By this means the killing concentration of the salt may be effectively reduced, according to the amount of the insoluble body present, either to a stimulating concentration or to one in which the physiological effect is not apparent. They reasoned that the solid absorbed the salt from the solution and in this way produced an effect closely paralleling that of simple dilution.

It was determined to test the effect of solids in solutions of sulfuric acid. Maize was here used instead of wheat. First, the strength of this acid necessary to prevent the growth of maize seedlings was

[6] TRUE, R. H. and OGLEVEE, C. C., The effect of the presence of insoluble substances on the toxic action of poisons. Science N. S. **19**:421–424. 1904. Also BOT. GAZETTE **39**:1–21. 1905. In this connection see also DANDENO, J. B., The relation of mass action and physical affinity to toxicity, etc. Am. Jour. Sci. **17**:437–458. 1904.

determined as lying between *n*/2750 and *n*/3250.[7] Then a series of varying concentrations of this acid on either side of the toxic limit was carried out, placing clear sand, quartz flour, filter paper, and paraffin shavings in the solutions. In no case was the apparent death limit modified by the presence of these substances. The death limit was also determined for a solution of sulfuric acid saturated with calcium sulfate, and for the same solution with an excess of calcium sulfate, but the solid again had no apparent effect.

With copper sulfate solutions carbon black was found to decrease the toxic effect, just as the authors above cited found to be true for the solids with which they worked. In order to bring out the effect of the carbon black, copper sulfate in the proportions of one and five parts per million of copper was added to the nutrient solution above described; a portion of the solution thus prepared was shaken with carbon black and then filtered, and wheat seedlings were grown in the treated and untreated solution. This series constitutes Experiment VI. Twenty-four plants composed a culture and the experiment lasted twelve days. The results are given in Table III.

TABLE III.

DATA FOR EXPERIMENT VI.

Culture no.	Medium	Total transpiration in grams
1	Nutrient solution	216.3
2	Do. + 1 p.p.m. Cu	67.3
3	Do. + 5 p.p.m. Cu	41.5
4	As 2, but carbon-treated	140.2
5	As 3, but carbon-treated	138.7

The growth of the plants was proportional to their transpiration. It is evident that the carbon removed sufficient copper to render carbon-treated solutions much less toxic than the untreated ones. This is a direct corroboration of the work of TRUE and OGLEVEE, but with another solid, and in this experiment the filtering out of the solid removes any possibility of its having any effect directly upon the roots. The explanation of these authors seems to be correct, as far as copper sulfate is concerned. The failure in the present

[7] This determination had been made previously, but was repeated for the present work. See CAMERON, F. K., and BREAZEALE, J. F., Toxic action of acids and salts on seedlings. Jour. Phys. Chem. **8**: 1–13. 1904.

instance to get the same effect with sulfuric acid may be due to the fact that to be injurious this substance must be at a much higher concentration than is needed in case of the copper salt, while the relative amount of solute absorbed by solids is much greater in dilute solutions than in more concentrated ones. TRUE and OGLEVEE suggest this explanation for their failure to get marked improvement by the use of sand in solutions of phenol and resorcinol.

On account of the presence of toxic substances in distilled water as ordinarily prepared, from copper boiler and tin condenser, most workers with toxicity problems have used water redistilled in glass. In these laboratories the distilled water is often quite toxic to wheat seedlings, but its injurious effect is prevented if it is first shaken with carbon black or ferric hydrate and the solid filtered off. It is found that water so treated produces as good a growth of seedlings as does the most carefully prepared redistilled water.

It appears from these experiments (1) that extracts of certain soils are toxic to wheat seedlings in water culture, and that this toxicity is removed wholly or in part by carbon black, calcium carbonate, ferric hydrate, and other solids; (2) that the toxic substances of ordinary distilled water may be removed by ferric hydrate or carbon black; (3) that the roots of wheat seedlings give off substances which are toxic to themselves and that these substances can be made inactive by the presence of the last named solids in the culture medium; (4) that the presence of ferric hydrate and carbon black in the solution seemingly accelerates to a marked degree the development of roots, causing them to surpass the tops in growth.

The work here reported was done chiefly in the laboratories of the U. S. Department of Agriculture, Bureau of Soils, Washington, D. C. It was finished at the Rhode Island Agricultural Experiment Station, Kingston, R. I. I am indebted to Dr. F. K. CAMERON, and to Dr. B. E. LIVINGSTON, of the Bureau of Soils, for much valuable suggestion and advice.

U. S. DEPARTMENT OF AGRICULTURE,
Bureau of Soils,
Washington, D. C.

BRIEFER ARTICLES.

NOTES ON NORTH AMERICAN GRASSES. V.

SOME TRINIUS PANICUM TYPES.

BELOW are given a few notes upon certain species of Panicum described by TRINIUS from American material. The herbarium of TRINIUS, the father of modern agrostology, is deposited in the St. Petersburg Academy of Sciences. The curator of the herbarium, Dr. D. I. LITWINOW, very kindly sent, for deposit in the National Herbarium, portions of the specimens of the species mentioned in this article. It will be observed that three of the seven species had been described by earlier authors, while four have been recently described as new species. It must be said, however, that it would be impossible to identify any of these four species from the description alone. This is true of many of the earlier descriptions of the group *Dichotoma* of the genus Panicum, and on account of the difficulty of consulting the types of these species, it is in a measure excusable, on the ground of convenience, to describe unidentified species as new, without determining the application of the older names. Such painstaking and laborious comparison should not, however, be shirked by a monographer.

P. CHAMAELONCHE Trin. 1826, Gram. Pan. 242.

Palmare et minus. Panicula (semipollicari) lucidissima; Spiculis pusillis, obovato-oblongis, obtusiusculis, glabris; pedicellis hispidulis; Gluma inferiore flosculis triplo breviori 1-, superiore eosdem aequante 5-nervi; Hermaphrodito elliptico, mucronulato, laevi, neutrum aequante.

V. spp. Am. bor. (TRATTINICK, ex. coll. Enslini).

Plantula caespitosa, stricta, culmo tenuissimo, ramuloso. Folia lanceolata l. lineari-lanceolata, strictiuscula, semipollicaria, glabra; culmea et juniora subinvoluta, acicularia, breviora. Panicula pauciflora, tenera, prodit e summa vagina elongata, cujus folium eandem fere aequat. Flosculus neuter univalvis? Hermaphroditus albescens.

Label accompanying type specimen: "In Am. bor. s. dat. sine nom. ex. hb^{io} Enslini cl. Trattinick."

Specimen = *P. Baldwinii* Nutt. 1898. Scribn. Div. Agros. Bull. 11:43.

P. DEMISSUM Trin. Spec. Gram. **3**:319.

The type specimen is from Rio Janeiro. I have not seen this species from North America. It is mentioned here because the name occurs occasionally in the literature of North American grasses, and has been doubtfully applied to certain of our species.

P. ENSLINI Trin. 1826, Gram. Pan. 230.

Pedale. Panicula (subdigitali) contractiuscula, lucida; Spiculis subultra-linealibus, ellipticis, acutiusculis, pilosulis: pedicellis glabris; Gluma inferiore flosculis sub 4-plo breviori 1-, superiore eosdem vix excedente 7-nervi; Hermaphrodito oblongo, acutiusculo, laevi, neutrum fere aequante.

An *Panicum tenue* Mühlenb. (quod *Pan. liton* Schult. Mant. 2. p. 250) quaerit ill. N. AB ESENB. in litt.

V. spp. Am. bor. (TRATTINICK, e collect. Enslini.)

Culmus basi ramosus, ad paniculam usque vaginatus: vaginis arctis, fissura pubescentibus. Folia glabra: radicalia lanceolata-oblonga l. ovata, sesquipollicaria, lineas 4–5 lata; superiora lineari-lanceolata, duplolongiora, patentia. Panicula e summa vagina prodit radiis subcapillaribus, parum patulis. Gluma inferior epilis, superior mucronulata cum flosculi neutrius valvula inferiore pilis adspersa. Hermaphroditus albescens.

Label accompanying type specimen: "(an *Pan. tenue* Muhlb. quaerit NEES AB ES.) ab Enslino in Am. bor. l. dt. sine nom. cf. Trattinick Wiennae 1820."

Specimen=*P. equilaterale* Scribn. 1898, Div. Agros. Bull. 11:42.

Characterized by having the spikelets of *P. commutatum* but the leaves elongated and widely spreading.

P. FLORIDANUM Trin. 1835, Mem. Acad. Petersb. VI. 3^2:248.

The type specimen from Georgia is *Paspalum racemosum* Nutt.=*P. bifidum* (A. Bertol.) Nash, as has been generally recognized.

P. JEJUNUM Trin. 1836. Bull. Acad. Petersb. 1:76.

The type specimen from Louisiana sent by Hooker in 1835 is *Panicum hians* Ell.=*Steinchisma hians* (Ell.) Nash.

P. LANCEARIUM Trin. 1822, Clavis Agros. 234.

1179. Gr. miliaceum americanum, minus, panicula parva. *Pluk. Phyt.* p. 176. *Tab. 92* f. *6.*

Mor. p. 197. no. 15. Panicum lancearium *m.* (*de quo alio loco*).

Label accompanying specimen: "Plukn. Tb. 92 f. 6.? In Am. bor. ab Enslino l. dt. cl. Trattinick."

Specimen=*P. Nashianum* Scribn. 1897, Div. Agros. Bull. 7:79.

The specimen matches *Curtiss 4029* from Florida, the first specimen cited in the original description of *P. Nashianum*. Both have glabrous spikelets. The second specimen cited by SCRIBNER, *Nash 466*, from Florida (the type on account of the specific name), has pubescent spikelets.

Since TRINIUS gives a binomial to a plant described by PLUKENET and by MORRISON under a polynomial designation, PLUKENET'S plant is the type.

In Kew Index *P. lancearium* is cited as Agrost. Bras. 246. The name

is mentioned in that work (NEES, 1829, Agrost. Bras. 226) in a note appended to *P. parvifolium* Lam., where it is referred to *P. angustifolium* Ell. It is quite distinct from ELLIOTT'S species, however.

TRINIUS himself describes the species later as follows (1826, Gram. Pan. 223:)

Spithamaeum. Panicula (vix pollicari) lucidissima; Spiculis parvis, obovatis, glabris: pedicellis scabriusculis; Gluma inferiore flosculis triplo breviori enervi, superiore eosdem aequante 7-nervi; Hermaphrodito oblongo, acutiusculo, laevi, neutrum aequante.

V. spp. Am. bor. (TRATTINICK ex hb^io. Enslini).

Ima basi in ramos discedit simplices, tenues attamen firmos, satis multi-(sex-)folios. Folia lanceolata, pollicaria, sensim breviora, praesertim basin versus ciliatula. Panicula e summa vagina prodit satis pauciflora, linearioblonga. Flosculus neuter bivalis. Hermaphroditus albens.

Since PLUKENET'S figures of this and the next cannot be identified, TRINIUS' specimens should be taken as the substitute type of these two species.

P. LEUCOBLEPHARIS Trin. 1822. Clavis Agrost. 234.

1177. Gr. miliaceum americanum, majus, panicula minore. *Pluk. Phytogr. p.* 176. *Tab.* 92. *f.* 7. *Mant.* p. 95. (*excl. Syn. Sloan. ut. ipse Sloaneus monet*). *Citatur a Gronowio* (Virg. p. 12.) *ad* Pan. paniculatum floribus muticis; *sed quid illud? Figura bene convenit cum Panico quodam herb. notsr. ex Amer. bor.* (Pan. leucoblepharis *m.*) *praeter cilia foliorum elegantissima, rigidiuscula.— Synon. Recchii ap. Pluk. admodum dubium.*

Label accompanying specimens: "ab Enslino in Am. bor. l. dt. cl. Trattinick."

Specimen = *P. ciliatum* Ell. 1816. Sketch 1:126.

Like *P. lancearium* the name is founded upon a figure in PLUKENET and is further described by TRINIUS in Gram. Pan. 219. 1826.

Spithamaeum et minus. Panicula (ultrapollicari) lucida; Spiculis subparvis, obovato-oblongis, pilosulis: pedicellis scabriusculis; Gluma inferiore flosculis plus duplo breviore 3–5, superiore eosdem aequante 7-nervi; Hermaphrodito oblongo, obtusiusculo, scrobiculato-punctato, neutrum aequante.

V. spp. Amer. bor. (TRATTINICK e plantis Enslini).

Basi ramosum. Folia cordato-lanceolata, amplexicaulia, pl. min. pollicaria, pallide viridia (plerumque elegantissime), pectinato-ciliata. Vaginae superiores elongatae. Axis pilosus. Gluma inferior epilis. Flosculus neuter bivalvis.

P. ciliatum is characterized by the ciliate but otherwise glabrous leaves and pubescent spikelets a little less than 2 ^mm long.

P. UNCIPHYLLUM Trin. 1826, Gram. Pan. 242.

Spithamaeum-pedale. Panicula (1-sesqui-pollicari) lucidula; Spiculis mini-

mis, oblongis, pilosis: pedicellis glabriusculis; Gluma inferiore flosculis triplo breviori 1-, superiore eosdem aequante 7-nervi; Hermaphrodito elliptico, laevi neutrum aequante.

Panicum laxiflorum Spreng. in Mém. de St. Pétersb. II. p. 291.

Panicum heterophyllum Mühlenb. teste N. ab Es.

V. spp. Am. bor. (TRATTINICK).

Culmus tenuis, adscendens, basi ramosus. Folia, quorum plura basi plerumque confertissima, lineari-lanceolata, cum vaginis pubescentia, pollicaria, lineas 2 lata: superiora angustiora, dissita. Panicula ovata, axis radiisque glabris. Flosculus neuter bivalvis. Hermaphroditus albescens.

Label accompanying type specimen: "Pan. heterophyllum Muhl. (Test. Nees) an Pluckn. Tab. 92 f. 8? ex herb Enslini, spmna Am. bor. Trattinick."

Specimen = *P. columbianum* Scribn. 1897. Div. Agros. Bull. 7:78.

In recent works this name has been applied to a species of the *lanuginosum* group having rather stiff foliage and the leaf blades hirsute on both surfaces. The true *P. unciphyllum* is easily recognized by the short crisp pubescence and the very short ligule, characters not mentioned in the original description.—A. S. HITCHCOCK, *U. S. Dept. Agric., Washington, D. C.*

SPOROGENESIS IN PALLAVICINIA.

THE August number of the BOTANICAL GAZETTE contains a paper by Mr. A. C. MOORE on *Sporogenesis in Pallavicinia*. I regret again to ask for space on this matter, but Mr. MOORE has so completely (though of course inadvertently) misrepresented my own position with regard to the nature and the significance of the quadripolar spindle in the Jungermannieae, and further, the grounds on which he founds his criticism appear to me to be so open to objection, that I venture to ask for an opportunity of replying to his strictures.

Firstly, then, as to the significance attached to the quadripolar spindle in 1894–5.

From Mr. MOORE'S account it would seem that I regarded, as the most essential feature of its importance, the simultaneous distribution of the chromosomes of the dividing nucleus of the mother-cell to the four spores that are finally produced.

I certainly believed that in *Pallavicinia decipiens* such a distribution occurred, and that it resulted from the suppression of the period of rest normally intervening between the first and second maiotic divisions. In this I may be right, or further investigations may show that, in the species in question, I missed the binucleate stage, But this is really not the

essential matter at all. The result of my work published in 1895 went to show that in most forms there are two consecutive mitoses, the second, following more or less rapidly on the first, and I believed that in *P. decipiens* the brief interval might be so shortened as to have become practically obliterated.

But the circumstance that quadripolar spindles were shown by me to be plainly visible in properly fixed material of forms in which no such extreme telescoping of the normal sequence of events takes place, clearly proves that, whatever the significance of the quadripolar spindle may be, it certainly is not *essentially* related to a simultaneous distribution of the chromosomes amongst four daughter nuclei, and I never thought it was.

What I believed in 1895 (and I have seen no reason to materially alter my view), was expressed as follows: "The quadripolar spindle, then, is only a special case of ordinary karyokinetic phenomena; instead of two relatively large masses of protoplasm there are four distinct aggregations, one in every lobe, each exercising an independent strain, and the direction of the strains may continue separate to the very end of the process or not, according to the form and special circumstances of the cell."[1] I may perhaps add, that the principal importance of the phenomenon, in my view, lay in its bearing on the permanence of the centrosomes, at that time a widely accepted doctrine.

In the second place, Mr. MOORE seems to think that his observations on *P. Lyellii* vitiate the conclusions based on a study of *P. decipiens*. I venture to think they do nothing of the sort. It is clear that the two species differ in the form of their spore mother-cells to a marked degree, and also that this difference is exactly of a nature to account for the unequal persistence of the peculiarities of the spindle in the two cases. For the lobing of the spore mother-cell is so much less in *P. Lyellii* than in the other species, that it would be a matter for surprise if the quadripolar character of its spindle were so long retained.

I confess, however, that I should have expected centrospheres to be present at the stages represented in *pl. III, figs. 1–3* of Mr. MOORE'S paper. They are so obviously demonstrable in *Aneura pinguis* and in *Fossombronia pusilla*, the spore mother-cells of which resemble in their lobing those of Mr. MOORE'S plant.

One feels a little difficulty in repressing a suspicion as to the successful fixation of his material, a suspicion not dispelled by the further contemplation of *figs. 12* and *13*. They so faithfully depict preparations I have

[1] Annals of Botany **9**:508.

myself very often obtained when the fixation had been imperfect. It is, of course, easy in these plants to secure admirable preparations of the stages preceding and following on the maiotic divisions, but I am sure Mr. MOORE will agree with me as to the great difficulty encountered in successfully fixing the cell contents at this critical period. Personally, I have not found chromacetic acid (the fixative used by him) very successful, but obtained far better results with Flemming's solution and, if due precautions are taken, with acetic alcohol. The latter, in particular, has yielded results of especial excellence, owing partly, no doubt, to the relative rapidity with which it traverses the somewhat impervious cell wall.—J. B. FARMER, *Royal College of Science, London.*

REPLY.

PROFESSOR FARMER acknowledges that in 1894 he believed in the simultaneous distribution of the chromosomes to the four spores in *Pallavicinia decipiens*. His description stands as the only account of a process without parallel in the plant kingdom, and he must have realized its exceptional nature. The account became all the more remarkable when Professor FARMER'S own studies on a number of liverworts, published in the following year, showed two successive mitoses in the spore mother-cells as in other groups of plants. He acknowledges now that he may have missed the binucleate stage. This is precisely what I believe he did, but since I have not investigated *P. decipiens* I cannot assert that he did so. Now he states that this simultaneous distribution is really not the essential matter at all. Apparently the essential matter to him is his observation that several liverworts conform to the normal sequence of nuclear division during sporogenesis. Yet these conclusions, bearing as they do on *Pallavicinia decipiens*, served to emphasize the peculiarities of that account, and I feel confident that most, if not all, cytologists would pick out the description of a simultaneous distribution of chromosomes as the most essential feature of his paper of 1894.

I venture to think that botanists are not so much interested in the explanations which Professor FARMER may make of what he did or did not believe in 1894 and 1895 relative to the quadripolar spindle (which opinions they can form for themselves), as in the facts of sporogenesis in the liverworts. My study of *Pallavicinia Lyellii* is plainly a challenge of his account of *P. decipiens*, and together with Professor DAVIS'S work on Pellia, leads us to believe that the "quadripolar spindle" in all liverworts is a phenomenon of prophase followed by spindles of two successive mitoses, in essential agreement with the events of sporogenesis in other plants.

The reader must judge for himself whether it is at all likely that two species in the same genus should differ from one another so fundamentally as would appear from Professor FARMER'S description of sporogenesis in *Pallavicinia decipiens* and my own account of *P. Lyellii*.

Respecting the fixation of my material, I may say that I have no reason to think the penetration was not sufficiently rapid to fix the cell contents. Even with imperfectly fixed material my main conclusion is easily demonstrable, viz., that in *P. Lyellii* there are two successive mitoses in the spore mother-cell. Let us not lose sight of the main point at issue.—
ANDREW C. MOORE, *South Carolina College, Columbia.*

CURRENT LITERATURE.

BOOK REVIEWS.

The algal vegetation of the Faeröese coasts.

BÖRGESEN'S extremely interesting account of the algal associations on the coasts of the Faeröe Islands,[1] is one of the most important contributions to the ecological side of marine botany. The work is a description of conspicuous algal associations along a varied rocky coast line, particularly favorable to algal vegetation, and is illustrated by more than thirty very excellent plates and figures from photographs. The factors affecting the algal vegetation are discussed; such as temperature and salinity of the water, tides and currents, wave action, temperature and humidity of the air, and light. The littoral and sublittoral floras are described, both for exposed and sheltered coasts, and also the floras of tide-pools and caves. A great many algal associations and formations may be clearly recognized in the Faeröes, some of them very conspicuous, as the Chlorophyceae formation, the Porphyra association, Fucaceae formations, Laminariaceae formation, and Alaria association. A particularly interesting chart plots the position of these associations in their position above and below the mean sea level.

It is extremely interesting to note that the cave flora is composed of forms of the sublittoral flora, which in the dim light are able to grow near the surface, or they are types which have the habit of growing in shaded situations outside. Littoral forms which grow in the brightest light are only found near the entrance of the caves. On entering a cave a condensed picture is obtained of the vertical distribution of algae from above downward. The forms in the deepest shadows are all red algae and some of them species which are usually found at great depths in the open sea. It is clear that light is the most important factor affecting the position of algal associations along a coast.

There is a detailed comparison of the algal flora of the Faeröe Islands with neighboring countries, Scotland, the Orkney and Shetland Islands, Norway, and Iceland, preliminary to a discussion of its origin. The flora had its origin from a mixture of Atlantic and Arctic species, which wandered northward with the retreat of the ice. Some of the arctic forms remained, adjusting themselves to the warmer waters, but there are many peculiarities of the algal flora which demand special explanations. BÖRGESEN does not believe that there were post-glacial bridges of land which made possible the migration of forms, but holds that factors now operative might have brought to the islands many algae from neighboring countries.

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[1] BÖRGESEN, F., The algal-vegetation of Faeröese coasts. Imp. 8vo. pp. 681–834. *pls. 13–24.* Copenhagen: H. H. Thiele. 1905. [Reprinted from Botany of the Faeröes. See BOT. GAZETTE **36**:392. 1903.]

Sea currents are regarded as of greatest importance. The pronounced currents from the nearest land do not bathe the islands, but experiments have proved that heavy winds and storms will drive floating objects out of the main currents, and BÖRGESEN believes the general conditions to be favorable to the introduction of algae from the west and north coasts of Ireland, the west coast of Scotland, and the Hebrides, while the currents from east Iceland run straight to the Faeröes. It is also possible that algae may be introduced from the west coast of Norway. Fragments of the algae may drift for many days, especially such as are provided with bladder-like floats, or their spores may be so carried, and floating pieces of timber covered with algal growths are known to travel long distances. Smaller algae of the littoral flora are very likely to be introduced with mud upon the feet and bodies of birds. Finally BÖRGESEN believes that algae may be introduced through the shipping which visits the islands.

These are merely some of the most striking conclusions in an account that is full of interesting observations on the life conditions and habits of marine algae.—B. M. DAVIS.

Plant diseases.

FREEMAN has produced a finely illustrated volume on plant diseases,[2] the first part of which is devoted to a discussion of fungi in general, while the second special part treats of specific fungous diseases of plants. The object of this book, as set forth in the preface, is "rather educational than immediately practical." It is an attempt to give a general account of the nature of fungi, for the purpose of encouraging study on the part of farmers and horticulturists rather than the habit of dependence upon rules and formulae. On this account the scope of the work becomes rather broader than would be indicated by the title, *Minnesota plant diseases.*

The first part comprises a discussion of the morphology, physiology, and ecology of fungi; but, while this part contains much excellent material, the arrangement lacks the logical sequence of first importance in a book of an educational character. It consists rather of a series of interesting pictures without due regard to pedagogical principles. This is likely to leave the mind of the reader confused. The sub-headings of the first chapter on nutrition are as follows: What the fungi are; The fungus method of obtaining nutrition; How the nutritive method is expressed in structure; Parasitism and saprophytism; Storage organs; Fungus shoestrings or strands; Physiology of the mycelium. Then, in chapter III, Fungus life methods, we have as the first subhead, again, Parasitism and saprophytism, the rest of the chapter dealing with habits or rather habitats of different fungi. Too great an effort is made to avoid scientific terms. Thus we have such confusing combinations as "spore-like swimming-spore-cases," "Sac-spore-capsule." It would seem that the reader who can comprehend the allusions to the phylogenetic relationships between fungi and algae would not find it too difficult to comprehend a few scientific terms.

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[2] FREEMAN, E. M., Minnesota plant diseases. Imp. 8vo. pp. xxiii+432. *figs.* 211. St. Paul: Report of the Survey. Bot. Ser. V. 1905.

The second part of the book is devoted to descriptions of special diseases. These are classified according to the nature of the crops on which they occur, as follows: Timber and shade trees; Field and forage crops; Garden crops; Orchards and vineyards; Greenhouse and ornamental plants; Wild plants. Under those heads the groups of fungi, as rusts, smuts, mildews, etc., are kept together. —H. HASSELBRING.

Regeneration.

WITH THE TITLE *Studies in regeneration* NĚMEC[3] has published in rather voluminous form the results of his investigations on the regeneration of root-tips. The general conclusions may be briefly summarized as follows. Cutting a transverse section just at the tip results in the regeneration of a new tip in a radial manner. The dermatogen and outer part of the periblem takes no part in this, the new tissue arising from the inner part of the cortex and the plerome. There is first of all the formation of a callus of hypertrophied cells, between which and the meristem arises the group of initials by which the new root-tip is organized. This group is radial from the beginning, the majority of its cells arising from the plerome, only the peripheral ones coming from the periblem. Proceeding back from the tip, the capacity for regeneration diminishes from the periphery inwards, soon disappearing from the periblem. As long as the central cells of the plerome still possess this capacity the regeneration is radial. Farther back it is confined to the pericambium and cells of the periblem and plerome immediately adjoining, which give rise to a ring of meristem, out of which usually more than one root-tip arises. When the capacity of the inner cells of the periblem and the outer cells of the plerome to take part in regeneration is lost, the replacement of the removed root-tip occurs only through the origin of lateral roots, which arise in the pericambium.

When the root is cut through obliquely, the regeneration of the new root-tip occurs at the part of the cut surface nearest the tip. When the tip is slit lengthwise each half re-forms a new tip. If a tip is slit lengthwise for about 1^{mm}, and then one of the halves is removed by a transverse incision, the remaining half regenerates a new half, and also, at the surface formed by the transverse cut, a new tip is developed. Lateral incisions to produce new roots must go at least half way through the plerome. Unless such an incision is made just back of the tip a new tip is soon organized immediately above the cut. The original tip is pushed to one side and finally is displaced entirely. When the incisions are made on two opposite sides of the root at different levels, new root primordia arise at each place, but only the one nearest the original tip continues to develop. If two incisions are made, on opposite sides and at the same level, a new root arises at each, but one is soon suppressed, while the other develops and finally replaces the original tip. About forty-eight hours after the wounding, starch usually appears in the cells of the periblem just above the cut. The grains are not yet

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[3] NĚMEC, B., Studien über die Regeneration. Imp. 8vo. pp. 387. *figs. 180.* Berlin: Gebrüder Borntraeger. 1905. *M* 9.50.

mobile, and are aggregated about the nucleus. In about twenty-four hours more, however, they become statoliths and fall to the bottom of the cells. During this time the original tip has been losing its starch, and there is a period of from forty-eight to seventy-two hours in which the old tip has lost its starch and the new tip has none in a movable form. During this period the roots are ageotropic.

In ferns the root-tips do not regenerate. Tips cut off transversely just back of the apical cell are unable to organize a new one, though they may continue growing for several weeks. As the statolithic starch is in the root-cap, and this does not regenerate, such roots remain ageotropic.

Besides the discussion of the experiments, a number of chapters are devoted to a discussion of such topics as the influence of external conditions on regeneration, polarity and regeneration, growth and regeneration, purposefulness of regeneration, relation between geotropism and the presence of statocytes, and other interesting topics connected with regeneration.

As the root-tip regenerates from so many kinds of injuries that could never occur in nature Němec considers that at least in the great majority of cases the capacity could not have arisen because of its utility. The immediate stimulus, he thinks, does not lie among nutritive changes, or arise from the wound, but is a phenomenon of correlation, due to the breaking of the connection between the vegetative tip and the root meristem.—W. B. McCallum.

Plant histology.

Chamberlain has revised and rewritten much of his *Methods in plant histology*,[4] adding several new chapters, elaborating and in many instances shortening the processes. Several new formulae are given for killing and fixing. The paraffin method has been notably improved and the celloidin method has been treated at greater length. A method for embedding in soap is also given.

The new chapters deal with microchemical tests, free-hand sectioning, special methods, the use of the microscope, and micrometric methods involving the use of the camera lucida. A very important new chapter deals with methods of staining filamentous algae and fungi and mounting them in Venetian turpentine. An abstract of the methods of Pfeiffer and Wellheim is given, together with such modifications as have been found to give successful preparations. Delicate forms like Vaucheria can be carried through the stains and finally mounted in Venetian turpentine without showing the least trace of plasmolysis, and even if slight plasmolysis should occur it can be corrected by manipulation of the mounting medium. Preparations made by this method are exceedingly brilliant and show a wealth of detail not possible with other methods. For example, the two nuclei in zygospores of Spirogyra can be readily seen with a low magnification. The Venetian turpentine method, which gives preparations requiring no sealing and as hard and durable as balsam mounts, should almost entirely replace the glycerin method.

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[4] Chamberlain, Charles J., Methods in plant histology. pp. x+262. *figs.* 88. Chicago: The University of Chicago Press. 1905. Net $2.25; postpaid, $2.39.

Much attention is given to collecting and keeping material alive in the laboratory. KLEBS'S method of securing reproductive phases in algae and fungi is presented in a practical manner. Specific directions are given for making such preparations as are needed by teachers and by those who wish to get a comprehensive view of the plant kingdom from the lowest to the highest forms. The book will be useful to those who wish to keep in touch with modern microtechnique.—W. J. G. LAND.

Bibliographical index of North American fungi.

THE compilation of a bibliographical index of North American fungi by FARLOW[5] is one of the most serviceable tasks ever undertaken in the interests of American systematic mycology, and the publication of it by the Carnegie Institution one of its best contributions to the promotion of botany. The work is the outgrowth of an effort to bring together references to all North American species in the form of a card catalogue. This was begun in 1874, at a time when there was no complete record of the species known from North America. Within a few years of its inception Mr. A. B. SEYMOUR was entrusted with the details of this herculean labor, under Dr. Farlow's direction, and his painstaking fidelity is worthy of recognition.

It is the aim of the work to include all references having any bearing on the taxonomy of fungi occurring in countries north of the Isthmus of Panama, the scope of the original plan (which was restricted to the region north of Mexico) having been greatly extended, on account of the close connection of species from our southern border with those of Mexico, Central America, and the East Indies. References to works of purely morphological, cytological, and physiological interest have been excluded; so have purely popular accounts, unless they were of use in giving distribution of the species or in furnishing good illustrations. In nomenclature the work is conservative. The principle of adopting the oldest specific name has been generally followed. Where the vagueness of older descriptions has made it uncertain to what species they applied the writers have had no scruples in rejecting the older names.

The index itself is arranged alphabetically. The names are printed in boldface type, synonyms and cross references being in italics. The citations, arranged in chronological order under each name, follow the form adopted by the Madison Botanical Congress in 1893 and by Section G, A.A.A.S. in 1894. In many cases of confused synonomy, critical examinations were made of authentic specimens and the related literature. Notes of interest obtained thus are added under the species in question. The present part, which is part I of the first volume, includes names from *Abrothallus* to *Badhamia*.—H. HASSELBRING.

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[5] FARLOW, W. G., Bibliographical index of North American fungi. Vol. I, part 1. 8vo. pp. xxxv+312. Washington: Carnegie Institution. 1905.

MINOR NOTICES.

Japanese vegetation.—Professor MIYOSHI, of the University of Tokyo, has begun the publication of photogravures of Japanese vegetation,[6] to represent wild and cultivated plants and plant societies. Each picture is on a separate sheet of cardboard 20.5×27^{cm}, the size of the print being 16×23^{cm}. Accompanying the illustrations is a descriptive text in both English and Japanese. The author has not yet determined the number of plates to be issued. So far, two parts have appeared, part I containing eight plates of cultivated and semi-cultivated plants, and part II containing eight illustrations of the vegetation of the island of Nikko.

The illustrations are well chosen and well made. Among the most effective and characteristic are the long avenues of giant mountain cherry trees, gorgeous with their spring blossoms, the graceful bamboos bending beneath their burden of winter snow, and the forest vegetation around the Hannya waterfall. The descriptive text is precise, and interspersed by interesting remarks which show that the author has an eye for color and setting.

It is to be hoped that the series may be continued to give us many more illustrations of the flora of this interesting country.—F. C. NEWCOMBE.

A botanical cyclopedia.—An illustrated German dictionary of botanical terms has appeared under the editorship of CAMILLO K. SCHNEIDER,[7] with the assistance of a number of other German botanists. This volume of almost 700 pages presents much more than a list of definitions, for there are illustrated descriptions of the morphology and minute structures of organs, of the sort one would expect to find in a cyclopedia. The terms, of course, are those employed in the German language, and the work will not take the place, for the English or American botanist, of JACKSON'S excellent *Glossary of botanic terms*. —B. M. DAVIS.

NOTES FOR STUDENTS.

Chemotaxis of spermatozoids.—The chemotaxis of the spermatozoids of Isoetes has been studied by SHIBATA.[8] In *Isoetes japonica*, which was used for the study, the sporangia ripen in autumn. Microspores, sown in tap water in Perti dishes late in November, begin to germinate about the middle of January. The duration of the swarming movements of the spermatozoids is shorter than in the ferns, vigorous movements lasting only about five minutes; some movement of

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[6] MIYOSHI, M., Atlas of Japanese vegetation. With explanatory text. Tokyo: Maruzen Kabushiki Kaisha. 1905.

[7] SCHNEIDER, C. K., Illustriertes Handwörterbuch der Botanik. Imp. 8vo. pp. 690. *figs. 341*. Leipzig: Wilhelm Engelmann. 1905. *M* 16.

[8] SHIBATA, K., Studien über die Chemotaxis der Isoetes-Spermatozoiden. Jahrb. Wiss. Bot. **41**:561–610. 1905.

the spermatozoid, however, may continue for ten or fifteen minutes, and of the cilia for five minutes longer. PFEFFER'S capillary method was used in the experiments. The principal headings are: position chemotaxis, relation between the intensity of the stimulus and extent of the reaction, repulsion by free acids and alkalis, negative chemotaxis with the ions of heavy metals, repulsive effect of alkali salts, behavior with osmotically acting substances, repulsive effect of ions of certain organic acids, the action of narcotics, theoretical, and review.

Malic acid acts as a strong topochemotactic stimulus and may be regarded as the specific stimulant for the spermatozoids of Isoetes, although certain other substances also exert some topochemotactic influence. Free malic acid in weak solutions exerts a positive chemotactic influence, but in stronger solutions a negative one. The salts of various metals act as negatively chemotactic stimuli, as do also the anions of di- and tribasic organic acids, including malic acid. The positive chemotaxis with malic acid is of a typically topotactic nature. The reaction consists in a turning of the body axis of the spermatozoid and a movement toward the source of stimulation. Whether the structure for the perception of chemotactic stimuli consists of the whole body of the spermatozoid or only of localized portions of it is not yet determined.—C. J. CHAMBERLAIN.

Tuberization.—The causes of tuberization still furnish a field for study. BERNARD first supposed that *Fusarium Solani* was the endophytic fungus of the potato; this has since been disproved by GALLAUD and by BERNARD himself, but the identity of the fungus is still undetermined. H. JUMELLE[9] has been conducting experiments on *Solanum Commersoni*, a tuber-bearing species related to the potato, but as yet his results are largely negative. The chief interest attached to his studies are occasioned by the fact that *S. Commersoni* has small slowly developing tubers placed on long stolons; these are the very characters which the potato is said to have had when first introduced into Europe, before the endophytic fungus became sufficiently abundant. *S. Commersoni* was infected by fungi from *S. tuberosum*, but, as stated above, with negative results. JUMELLE thinks that with suitable infection, it may be possible to secure tubers like those of the potato, and further experiments are in progress. It should be said that GALLAUD thinks that BERNARD has not yet isolated the true tuber-forming fungus.—H. C. COWLES.

Two parasitic fungi.—KLEBAHN[10] has worked out the life histories of two common species of the so-called Imperfecti group. The first of these is the common elm fungus, *Phleospora Ulmi* (Fr.) Wallr. This is connected with an ascomycetous form, which appears on the infected dead leaves during the winter and ripens in spring, when the spores are ejected and infect the young

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[9] JUMELLE, H., Del'influence des endophytes sur la tubérisation des Solanum. Rev. Gén. Bot. **17**:49–59. 1905.

[10] KLEBAHN, H., Untersuchungen über einige *Fungi imperfecti* unddie zugehörigen Ascomycetenformen. I. u. II. Jahrb. Wiss. Bot. **41**:485–560. *figs. 75.* 1905.

elm leaves. The form is named *Mycosphaerella Ulmi* Klebahn[11]. Both conidia and ascospores produced identical mycelia in cultures. The Phleospora was produced by sowing ascospores on the under side of elm leaves. No infection took place from spores sown on the upper surface. The study of *Gloeosporium nervisequum* (Fckl.) Sacc. revealed a rather complicated series of forms belonging to this fungus. An ascogenous stage develops on the dead leaves, as in Phleospora. This is *Gnomonia Veneta* Klebahn. Beside the usual conidial form and the ascogenous form, the fungus assumes the form of a Myxosporium on the young branches, and there produces the twig wilt always noticed on sycamore trees affected with the Gloeosporium. A fourth form develops on the dead leaves. This is a conidial form of the Fusicoccum type. Proof of the connection of all these forms rests mainly in the similarity of the mycelia produced in pure cultures from the various spore forms. Infections could not be produced readily, but a few cases of inoculation with ascospores were successful. The various spore forms have been described under different names, which are given in the synonymy.—H. HASSELBRING.

Shore formations in Denmark.—WARMING, in collaboration with WESENBERG-LUND and others in an interesting paper, has correlated the work of plants and animals in the shore formations of western Denmark.[12] A "vad" is a shallow coastal lagoon, cut off from the sea proper by a line of islands, and bare at low tide; the bottom may be of sand or clay, the latter type prevailing in the more tranquil places. The sandworm, *Arenicola marina*, is the most characteristic animal of the sandy "vader" or shallows, and the excrements of this worm are found there in great abundance. Hence it has commonly been thought that these animals have a soil function, similar to that of the earthworm, and that they help to build up the shallow into a marsh. The authors, however, find that Arenicola is very sedentary in its habits, swallows only from surface layers, and that it retards rather than furthers soil enrichment. The waves wash any fine particles landward, leaving the Arenicola shallow as sandy as before and hence well adapted for the continued prosperity of Arenicola. In shallower water closer to the shore, where the bottom material is finer, the amphipod, *Corophium grossipes*, dominates; here the bottom is characteristically red-brown in color and presents a riddled surface appearance. The Corophium shallows teem with animal life, and here the influence of the animals is such as to convert the area somewhat rapidly into a marsh. To this work blue green algae contribute something, but animals are much more important land builders than plants in the Corophium shallows. Large areas of land have been gained from the sea in this way in Denmark, and one case is cited where a fertile meadow has been developed from a barren sandy shallow within two hundred years. In the argillaceous

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[11] Preliminary note in Zeitschr. Pfl. krankh. **12**:257. 1902.

[12] WARMING, E., Bidrag til Vadernes, Sandenes, og Marskens Naturhistorie. In collaboration with WESENBERG-LUND, ÖSTRUP, et al., with French résumé. Kongl. Dansk. Vid. Selsk. Skrift. VII. **2**:1–56. 1904.

shallows, in contrast to the above, plants are the more efficient land-builders; the developmental processes in such places are well known and need not be recounted here. WARMING also speaks of sandy plains that are subject to occasional inundation. Here algae play a great part in soil-making; it is common for a layer of Phycochromaceae to penetrate for three to five centimeters into the sand, cementing the grains together, and giving a greenish appearance to the ground. Many of the diatoms characteristic of such places are listed by habitats. The peculiar depressions of salt marshes, called "pans" by OLIVER and TANSLEY, are thought by WARMING to be formed where heaps of decaying vegetation have lain; the consequent destruction of the vegetation makes it easy for the waters to wash out the soil in such places.—H. C. COWLES.

Periodicity of sexual organs in Dictyota.—WILLIAMS, in the third contribution to his series of *Studies in the Dictyotaceae*,[13] discusses the remarkable periodicity in the formation of the sexual cells in Dictyota. The sexual organs are produced during the fruiting season in fortnightly crops, synchronous with the spring tides, and a general liberation of the gametes takes place on a particular day, at a fixed interval after the highest spring tide, varying, however, in different localities. Of the factors (temperature, pressure, aeration, etc.) which fluctuate with the alternation of neap and spring tides, the one which seems to account most satisfactorily for the facts of periodicity is the increased illumination of the plant during the low water of spring tides. The times of initiation and liberation may be slightly accelerated or retarded by exceptional meteorological conditions, as when wind causes a difference of two or three feet in the height of water, or a rise of one inch in the barometer accompanies a depression of six or seven inches in the tide.—B. M. DAVIS.

Brown pigment of algae.—The generally accepted view that the color of the chromatophores of the brown algae and diatoms results from the presence of a brown pigment, phycophaein, in addition to chlorophyll, is challenged by MOLISCH,[14] who believes that the phycophaein extracted from these algae is a post mortem product. He holds that the brown pigment is a single substance, phaeophyll, which passes readily over to chlorophyll when treated with hot water, alcohol, and other fluids. A similar brown pigment is found in the orchid, *Neottia nidus-avis*. Beside the phaeophyll, the brown algae and diatoms contain carotin and a bluish-green pigment, leucocyan. —B. M. DAVIS.

Bracts of Bennettites.—LIGNIER[15] from a reexamination of his preparations of the involucral bracts of *Bennettites Morierei* concludes that in all the sterile scales, superficial or otherwise, which enter into the composition of the strobilus, the terminal enlargement is due to hypertrophy and does not result from a reduction of the bract.—W. J. G. LAND.

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[13] WILLIAMS, K. L., Studies in the Dictyotaceae. III. The periodicity of the sexual cells in *Dictyota dichotoma*. Ann. Botany **19**:531–560. *figs. 6.* 1905.

[14] MOLISCH, H., Ueber der braunen Farbstoff der Phaeophyceen und Diatomeen. Bot. Zeit. **63**[1]:131–162. *pl. 6.* 1905.

[15] LIGNIER, O., Notes complémentaires sur la structure du *Bennettites Morierei*. Bull. Soc. Linn. Normandie V. **8**:(pp. 7.) 1904.

NEWS.

PROFESSOR EDUARD STRASBURGER traveled in Egypt during part of December and January.

DR. OSKAR BREFELD, professor of botany at Breslau, has retired owing to failing eyesight.

MR. WALTER FISCHER has resigned his position as Assistant in Botany at the Ohio State University, and has taken up work in the United States Department of Agriculture, Bureau of Plant Industry.

DR. A. F. BLAKESLEE, who is spending the winter in investigations at Halle, was awarded the Bowdoin prize of Harvard University ($200) last spring for his work on sexual reproduction in black molds.

IN November and December Dr. JOHN W. HARSHBERGER, of the University of Pennsylvania, delivered a series of five lectures on "Weird and marvelous plants" in the Ludwick Institute courses of free lectures, Philadelphia.

DR. ERNEST A. BESSEY has been transferred from Washington to the Sub-tropical Laboratory of the U. S. Department of Agriculture at Miami, Florida, where he may be addressed in future. Professor P. H. ROLFS, formerly of the Sub-tropical Laboratory has accepted the directorship of the Agricultural Experiment Station of Florida.

A RECENT circular gives an account of the Royal Hungarian Central Institute of Viticulture, the buildings of which were completed in 1904. This institute was initiated in 1896 by a law decreeing the establishment near Budapest of an institution for studying the problems of viticulture and wine-making, and giving scientific and practical instruction in these subjects. In 1898 Dr. ISTVÁNFFI, then professor at the University of Kolozsvar, but perhaps better known from his association with BREFELD during part of the latter's extensive investigations, was called to organize and direct the new institution, whose first work was done in quarters rented until the completion of the new ones. The present buildings are five, each of three stories. The main hall, 66×21^m contains the library, museum, offices, and lecture room. The four smaller halls, all similar in construction, are devoted respectively to the four sections: (1) physiology and pathology, (2) chemistry, (3) zymology, and (4) practical viticulture and oenology. The institution is excellently equipped for carrying on these different branches of work. Its primary object is that of an experiment station whose field is restricted to the wine-growing interests alone; secondarily, provision is made for instruction to advanced students in the practical aspects of viticulture and oenology.

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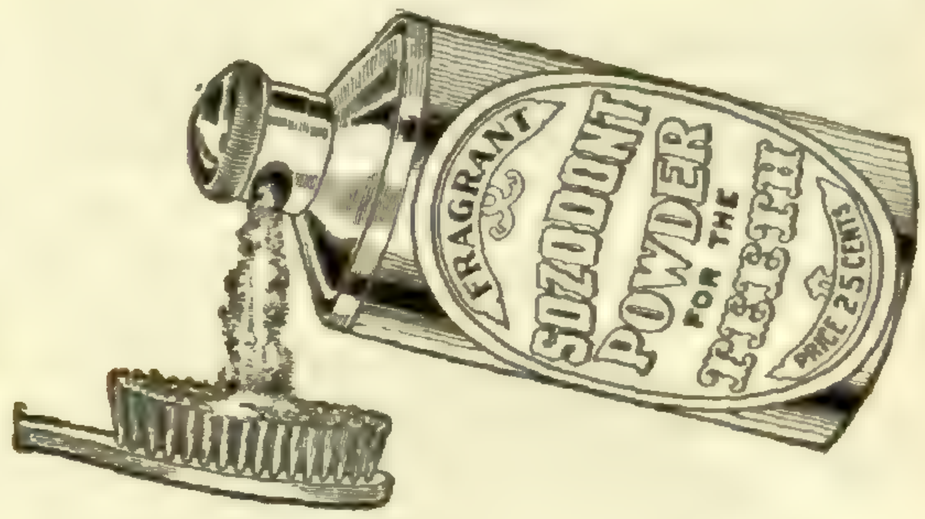

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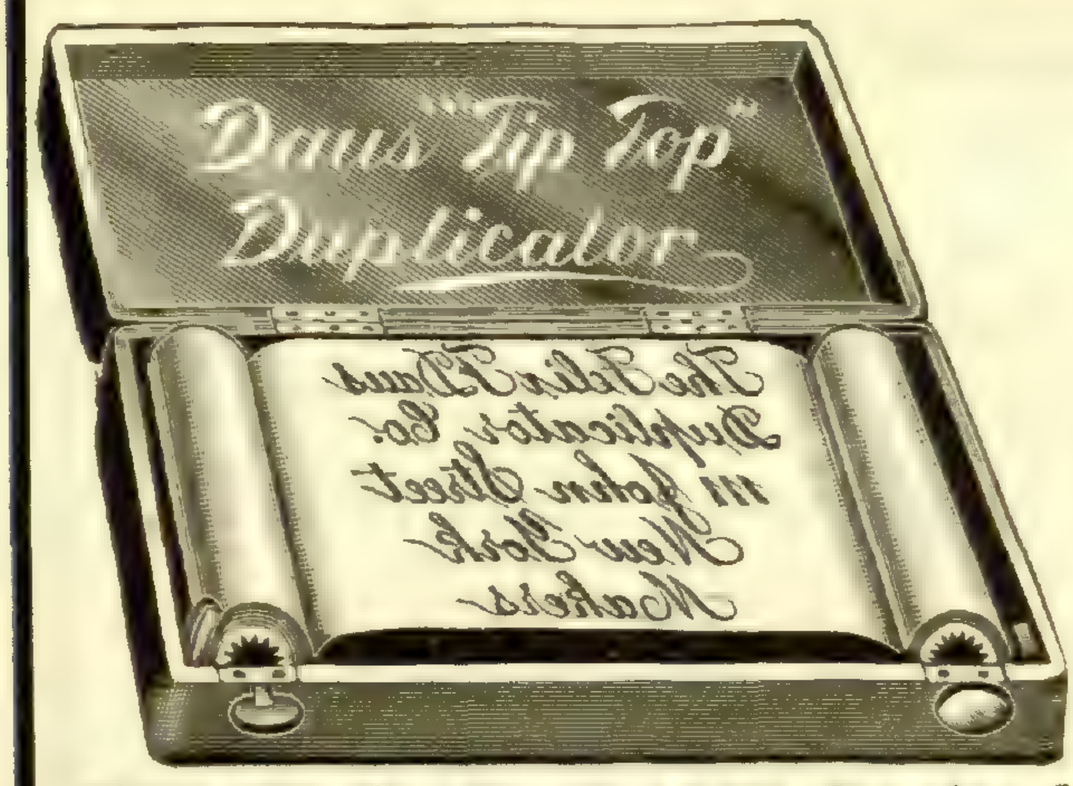

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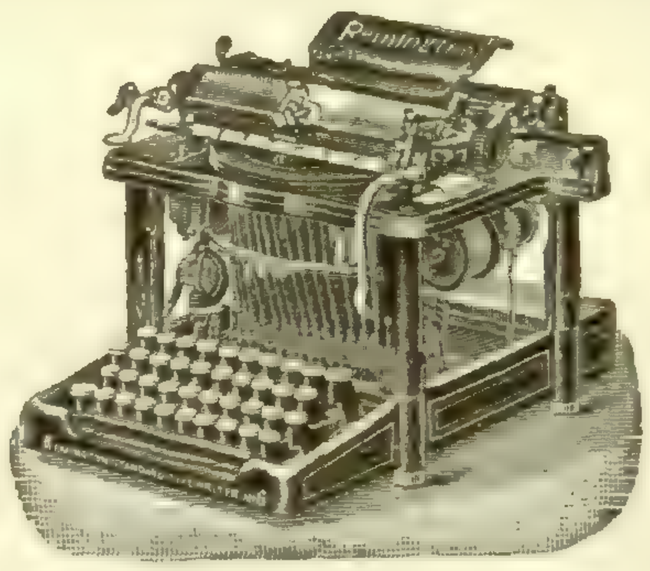

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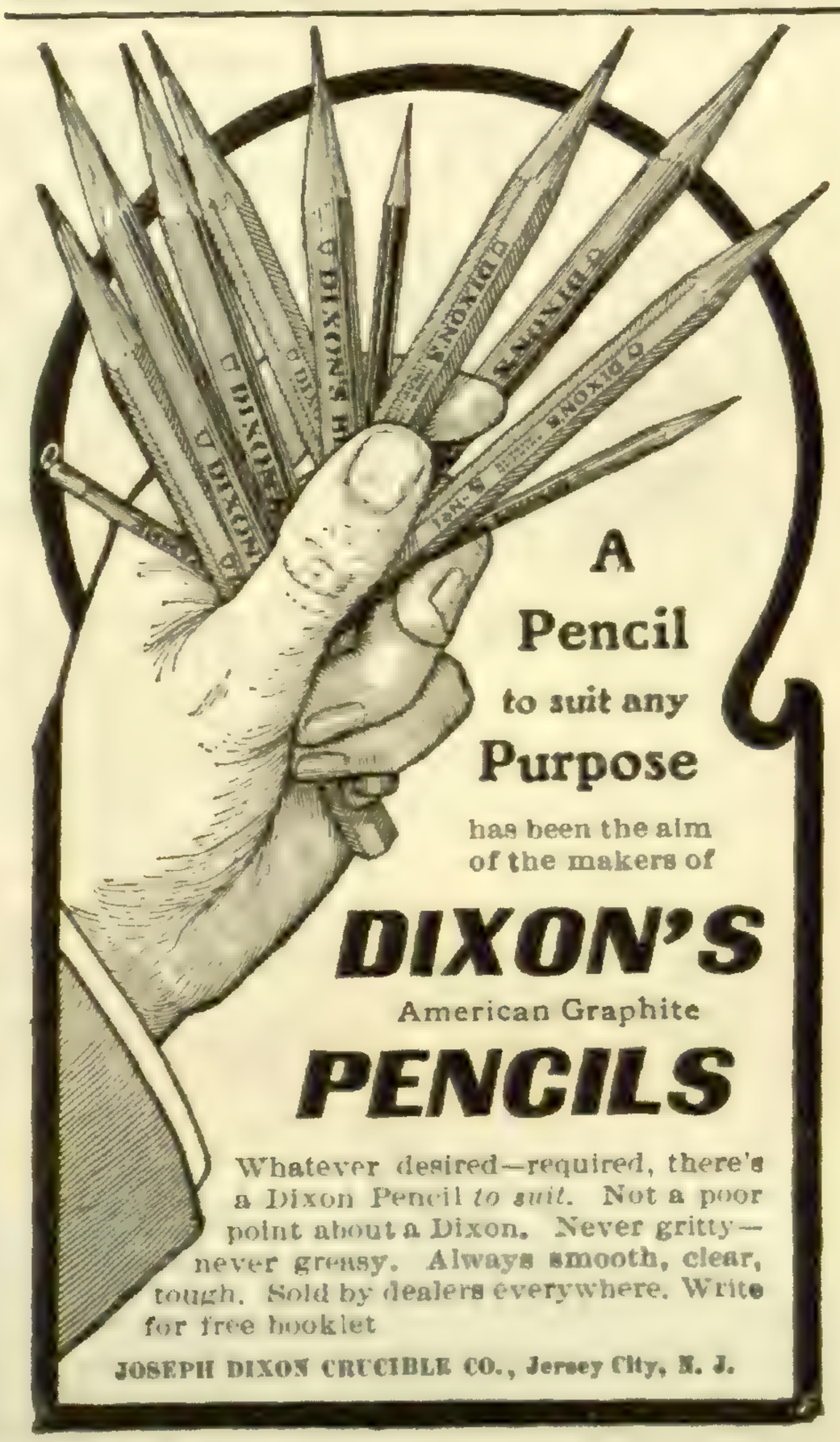

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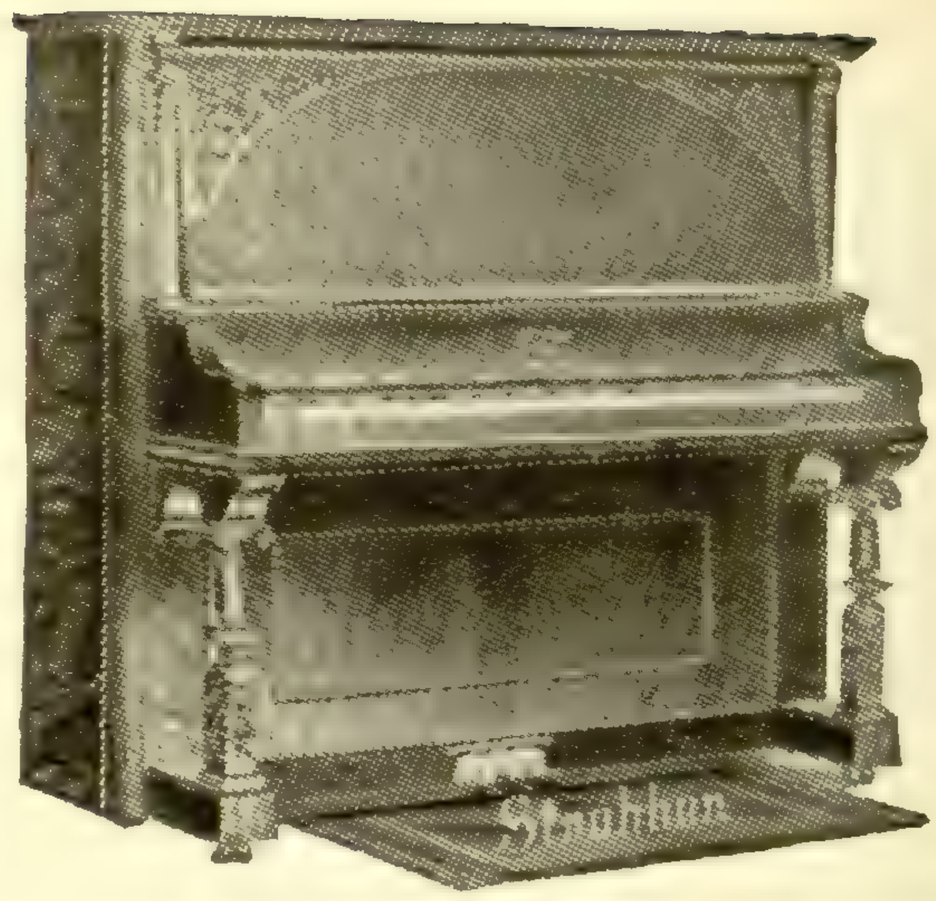

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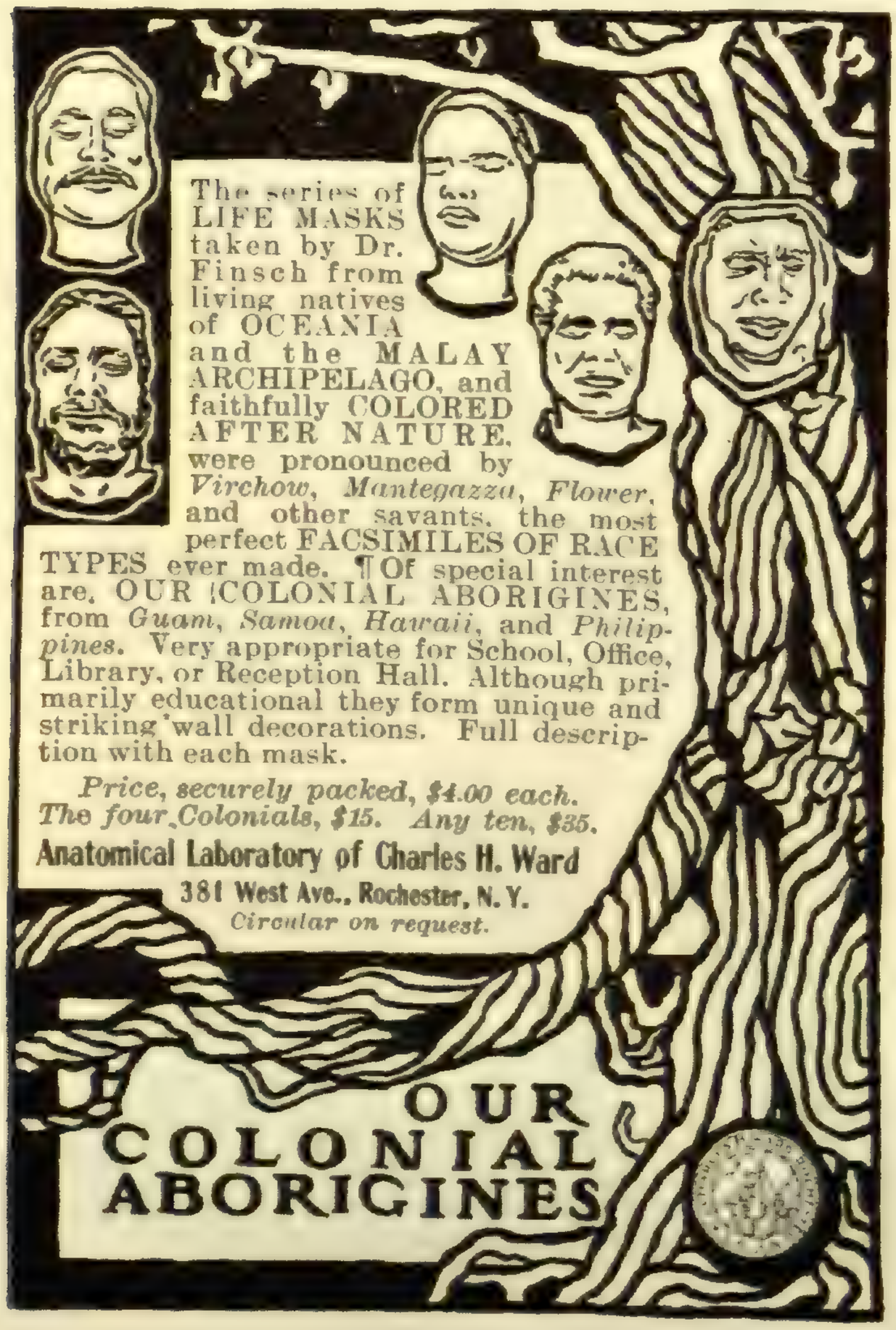

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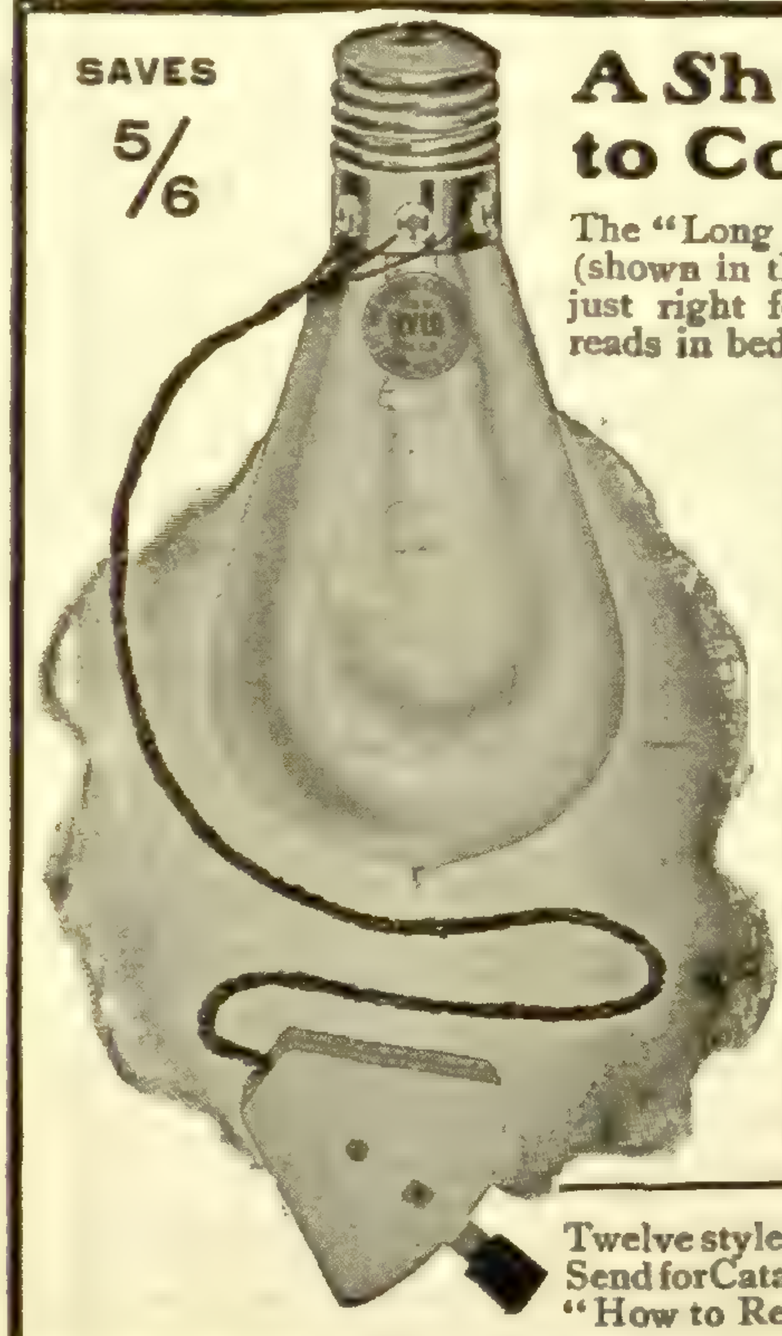

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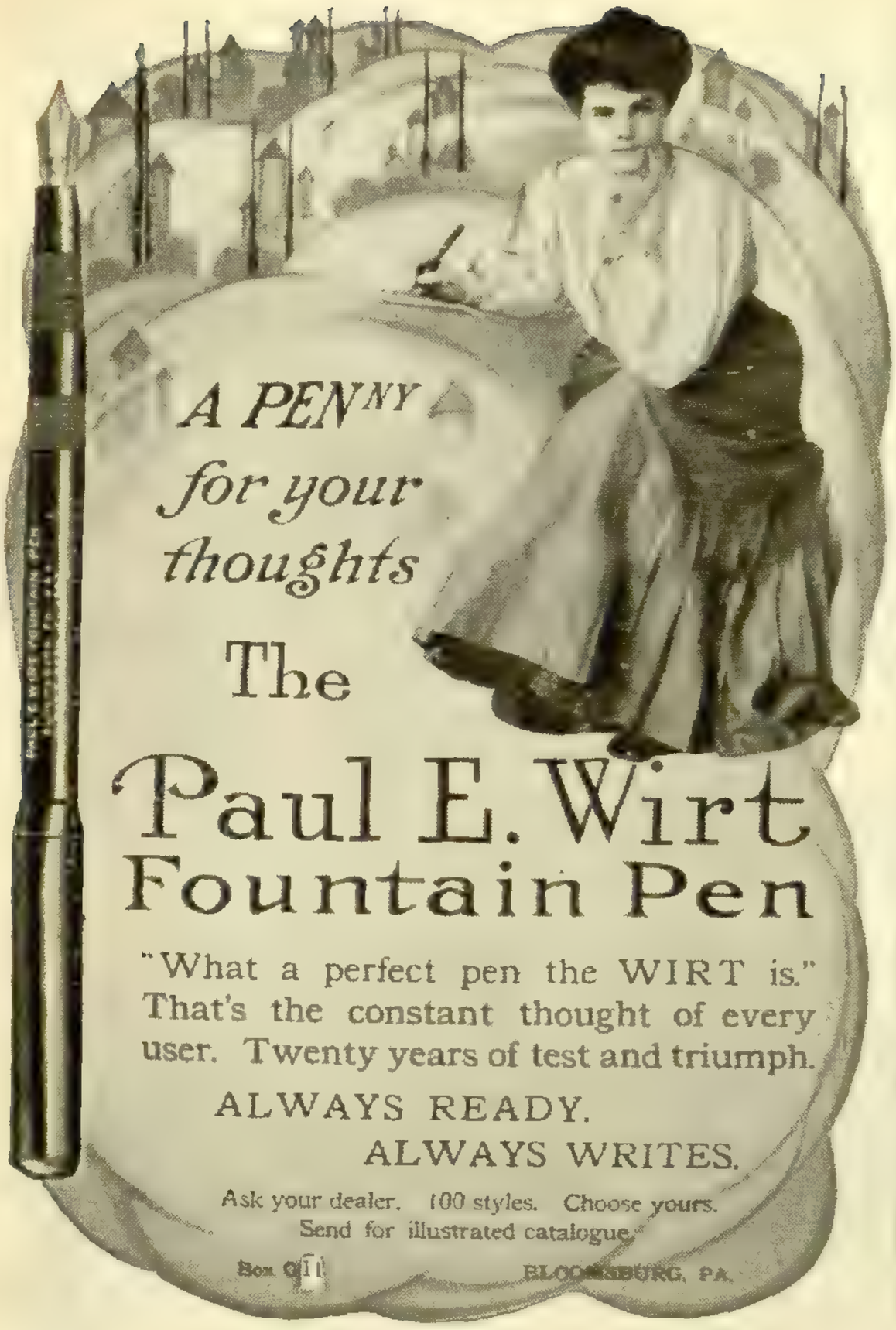

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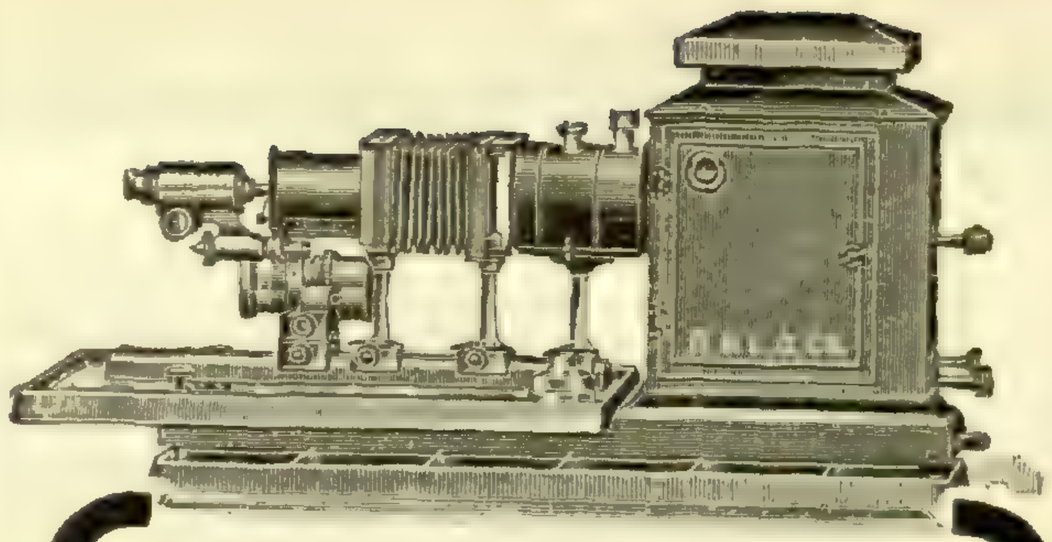

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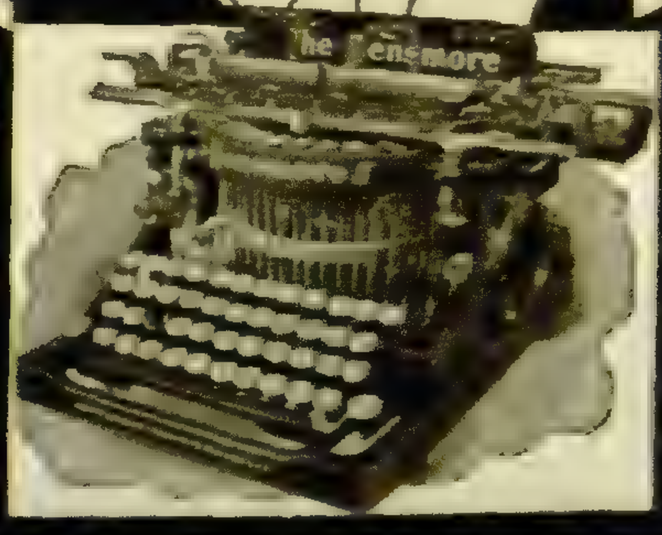

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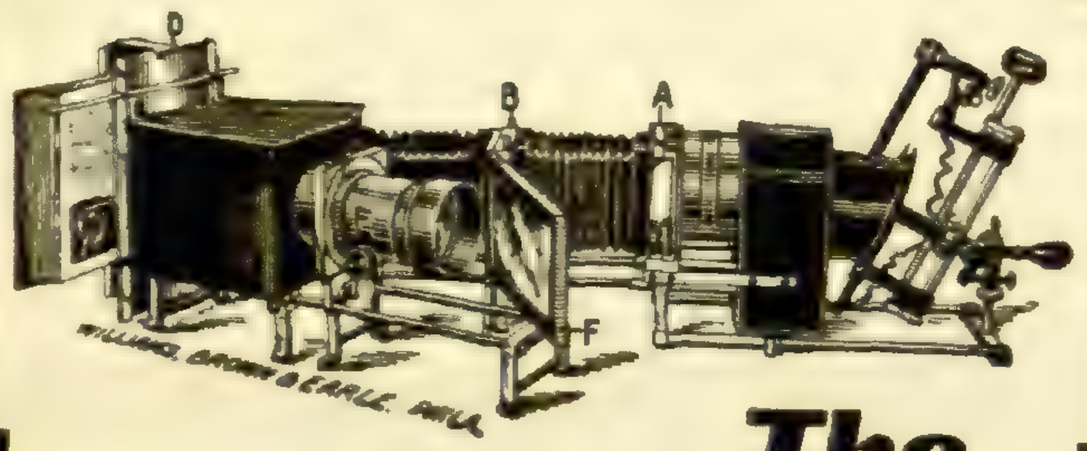

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In preparing the second edition the author has kept in view the advance in the science since the book first appeared. Professor Klebs's methods for securing various reproductive phases in the algae and fungi have been outlined in a practical way, and in general much more attention has been given to collecting material. New chapters deal with the Venetian turpentine method, micro-chemical tests, free-hand sections, special methods, and the use of the microscope. These changes and additions have enlarged the volume from 168 to 272 pages.

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BOTANICAL GAZETTE

FEBRUARY, 1906

CHEMOTROPISM OF FUNGI.[1]

HARRY R. FULTON.

HISTORICAL.

DE BARY (**5**) advanced the supposition that the oogonia of certain Phycomycetes not only attract the antheridia-producing branches, but determine the formation of these branches as well. The same writer (**6**) later raises the question as to whether the bending of a germ-tube toward the epidermis of its proper host, but not toward every membrane or moist surface, may not be brought about by a specific reaction in the parasite, induced by physical or chemical stimuli which may be supposed to operate through unknown secretions from the host plant. This was written, though not published, before PFEFFER'S first studies (**25**) on the reactions of motile unicellular organisms to chemical stimuli. During the progress of his studies with Saprolegnia swarm-spores, he observed that the hyphae of the fungus turned toward the nutrient substances, and he supposed that chemical agents might in many cases determine the direction of growth of these and other hyphae.

KIHLMAN (**16**) observed that if a germinating ascospore of *Melanospora parasitica* lies at a distance of not more than four or five spore-diameters from a growing hypha of its host, *Isaria farinosa*, the direction of growth of the latter is deflected toward the spore of the parasite until there is contact with its germ-tube.

According to BREFELD (**1**), the fact that neighboring sporidia of Ustilaginaceae conjugate in pairs, the connecting tubes taking the shortest course between two sporidia, may be explained by assuming a directive chemical influence.

[1] Contribution No. 4 from the Botanical Laboratory of the University of Missouri.

WORTMANN (**36**) observed the turning of young germ-tubes of *Saprolegnia* sp., and concluded that these are very sensitive to chemical stimuli, especially to those concerned in nutrition; a most energetic turning was observed toward flies' legs.

MARSHALL WARD (**32**) mentions two factors as mainly influencing the direction of growth of "lily-botrytis," namely, the contact of hyphae with one another or with solid bodies, and the direction in which food lies in relation to the growing hyphae.

WORONIN (**34**) holds that it is through chemical influence that the conidial germ-tubes of *Peziza baccarum* reach the wounds of the host plant.

STRANGE (**29**) made careful observations on growing hyphae of *Saprolegnia ferax* for the purpose of confirming their reported turning toward nutrient substances. That the hyphae turn toward a region of diffusing nutriment was regarded as very questionable; there was noticed, however, a stronger growth of the hyphae in the nutrient region, and the hyphae, by their branching, became more abundant here than elsewhere. The germinating conidia sent their tubes in all directions, provided enough nutriment for growth was present, and not markedly toward any area containing a greater amount of nutriment. Similar results were obtained with germ-tubes from Penicillium spores; these showed no chemotropic turning, but a much better growth when they reached the diffusion region around the openings of the capillary tubes containing the test solutions.

REINHARDT (**28**) found that the direction of growth in *Peziza* sp. is affected by chemical influences. Gelatin containing sugar and spores of *Mucor* sp. have an attractive effect. Spores of *Aspergillus niger*, *A. flavus*, and *Penicillium glaucum*, as well as colonies of various bacteria, cause a cessation of growth, which is followed by a reversal of the direction of growth.

BÜSGEN (**2**) observed chemotropism in the case of *Botrytis cinerea*, and supposed that a chemical stimulus might cause the germ-tubes in the dew-drop to seek the host epidermis; but he considered that penetration is brought about by contact influence. He asserts the possibility of chemotropism in the case of germ-tubes from uredo-

spores and from the conidia of Peronosporaceae, and of chemotaxis in the case of Cystopus swarm-spores.

Many of the foregoing are merely opinions or passing observations, made in the course of investigation of other phenomena. MIYOSHI (**20**), however, made chemotropism of certain fungi the subject of systematic and extensive experimental study. The principal fungi used by him were *Mucor stolonifer*, *M. mucedo*, *Phycomyces nitens*, *Penicillium glaucum*, *Aspergillus niger*, and *Saprolegnia ferax*. The tests were made with the aid of perforated membranes, such as strips of epidermis, celloidin films, and mica plates; of capillary tubes; and of injected leaves and petioles of *Tradescantia* sp. He concludes that, in the case of the fungi enumerated, molecules of many substances diffusing from the openings cause diversion of the hyphae from their original direction of growth, the turning being either toward the diffusing substance (positive chemotropism), when the substance is attractive to the fungus, or away from the substance (negative chemotropism), when the substance is repellent. Some substances are wholly or almost wholly neutral. The direction and amount of turning are dependent upon the concentration. Chemotropism is most marked at an optimum concentration, which varies for the substance and the fungus. The concentration just sufficient to cause turning is very low for most attractive substances. Repellent substances are acids, alkalis, alcohol, certain neutral salts and toxic compounds; also very strong solutions of substances that are neutral or attractive at lower concentrations. Generally attractive substances are ammonium nitrate, ammonium chlorid, ammonium malate, ammonium tartrate, potassium phosphate, sodium phosphate, ammonium phosphate, meat extract, peptone, sugar, asparagin, etc. For chemotropic phenomena Weber's law holds. The effect of an attractive substance may be overcome by the presence, in sufficient quantity, of a repellent substance.

The same investigator (**21**), in connection with his study of the penetration of natural and artificial membranes by fungi, found that the hyphae of *Botrytis cinerea* and *Penicillium glaucum* would grow through a membrane only when they were placed on nutrient substrata; there would be, however, no penetration through the membrane to

the substratum if the fungi were grown in a nutrient medium, although the mycelial growth was more vigorous.

SWINGLE (**31**), in explanation of the effects of Bordeaux mixture, advanced the hypothesis, which he based on the studies of REINHARDT, BÜSGEN, and MIYOSHI, that copper hydroxid may prevent the germ-tubes of parasitic fungi from entering the host plant through negative chemotropic action.

NORDHAUSEN (**24**) accepted MIYOSHI'S conclusions, and investigated the biological bearing of chemotropism upon the penetration of plant tissues by certain fungi, without bringing forward any additional evidence in favor of chemotropism.

No further investigation seems to have been made with relation to fungi, until CLARK (**4**), in his investigation of SWINGLE'S hypothesis, had occasion to test the chemotropic reactions of certain species, especially *Mucor stolonifer*, to toxic substances. For the most part he followed MIYOSHI'S methods closely. In every case it was found that the hyphae would turn from a nutrient medium and grow into media containing such toxic substances as salts of copper, cobalt, nickel, zinc, etc., until a concentration sufficient to cause death was reached. The hyphae turned toward non-nutrient media and distilled water as readily as toward nutrient media. His conclusion is that *Mucor stolonifer* is negatively chemotropic to some secretion of its own mycelium, and that this negative chemotropism is much greater than any positive chemotropism it may have for food substances or oxygen.

MASSEE (**19**) found that fungi are attracted to their hosts by specific stimuli from substances in the cell sap. In the case of saprophytes and facultative parasites, the attractive substance is saccharose; the facultative parasites, however, may be repelled by more potent negatively chemotropic substances in the cell sap. In the case of obligate parasites, the cell sap of the host plant is the strongest positive chemotropic agent; malic acid is the specific attractive substance for the germ tubes of *Monilia fructigena*, and the enzyme pectase for *Cercospora cucumis*. Immune plants owe their immunity to the absence of the chemotropic substance.

Other factors affecting the direction of growth of fungous hyphae have received but little attention, while the causes of the bending

of sporangiophores, especially of certain Mucorineae, have been carefully studied.

A negative hydrotropism for the sporangiophores of *Phycomyces nitens* was first experimentally established by WORTMANN (**35**), and was later confirmed by the more extended studies of ERRERA (**10**) and of STEYER (**30**). MOLISCH (**22**) showed that the sporangiophores of *Mucor stolonifer* and *Coprinus velaris* are negatively hydrotropic; while KLEBS (**17**) made similar observations for *Sporodinia grandis*, which FALCK (**12**) has failed to confirm.

WORTMANN (**35**) observed what he supposed to be negative hydrotropism in the case of the mycelium of *Mucor* sp., which would grow towards water, but would turn aside and branch profusely on reaching its surface. The conditions of experiment were such as to make his explanation of the phenomenon doubtful. STEYER (**30**) concludes that moisture plays an unimportant rôle in determining the growth and spreading of the mycelium of *Phycomyces nitens*.

JÖNSSON (**15**) grew mycelia of *Mucor stolonifer*, *Phycomyces nitens*, and *Botrytis cinerea* on sloping filter-paper strips having their two ends dipped in vessels of water at different levels. Phycomyces and Mucor showed negative rheotropism under these conditions, while Botrytis showed positive rheotropism.

HOFMEISTER (**14**), WORTMANN (**35**), DIETZ (**7**), KLEBS (**17**), and STEYER (**30**) have established for various Mucoraceae a negative reaction of their sporangiophores to gravity and to strong light; but there is a positive reaction to contact and to moderate intensities of light.

KNY (**18**) holds that gravity has no effect upon the growth of the mycelium of *Mucor mucedo*, *M. stolonifer*, *Trichothecium roseum*, and *Eurotium repens*. MIYOSHI (**20**) concludes from his tests that neither gravity, light, contact, nor moisture affected the turning of the six fungi used in his investigations. STEYER (**20**) in his study of the reactions of Phycomyces, found that the mycelium is indifferent to light, contact, and gravity.

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FACULTATIVE SAPROPHYTES
- Sphaeropsis malorum
- Cercospora apii
- Monilia fructigena

FACULTATIVE PARASITES
- Botrytis vulgaris
- Daedalia quercina

STRICT SAPROPHYTES
- Mucor stolonifer
- Mucor mucedo
- Phycomyces nitens
- Penicillium glaucum
- Monilia sitophila
- Sterigmatocystis nigra
- Coprinus micaceus
- Agaricus fabaceus

With exceptions as noted below, spores of the various fungi from pure cultures one to two weeks old were used in making inoculations. *Sphaeropsis malorum* was not ready in pure culture as soon as needed, and inoculations for the first experiments with this form were made directly from infected apple twigs; the spores were found to germinate quickly and the hyphae grew rapidly, so that the observed bacterial and mold contamination in these cultures was slight. *Cercospora apii*, obtained from celery leaves, was grown in artificial culture on pieces of sugar beet; spores were not produced, but satisfactory inoculations were made with small portions of detached mycelium. Inoculations in the case of the three Hymenomycetes were made with portions of mycelium from pure cultures, which had been made from sporophores by the "tissue-culture" method (DUGGAR, **9**). Spores of *Uromyces caryophyllinus*, taken directly from carnation leaves, were used to some extent. The germination of these was not certain under all conditions, and the growth was limited; the use of the fungus was soon abandoned. All other spores gave perfectly satisfactory germination in gelatin and agar-agar media. Even such species as *Penicillium glaucum* and *Sterigmatocystis nigra*, which have been found (DUGGAR, **8**) not to germinate in distilled water, gave a germination of practically 100 per cent. in gelatin and agar made up with distilled water.

Precautions were taken to have all apparatus chemically clean and thoroughly sterile. Glassware and mica plates were boiled in alkali and in acid, and again, after a thorough rinsing, in distilled water. Covers were rinsed in 95 per cent. alcohol, then wiped with a sterile cloth. Heavier glassware and mica plates were sterilized with dry heat at a temperature of 140° to 150° C. Celloidin films were sterilized by being boiled in redistilled water; strips of epidermis, by being

rinsed in alcohol and afterwards soaked in two changes of sterile redistilled water. The media used were sterilized, whenever possible, under 15 lbs. pressure of steam, or by fractional sterilization at 100° C. Except in those experiments in which capillary tubes were used, contaminations were of rare occurrence; whenever contamination was apparent, the experiment was disregarded in tabulating results. With few exceptions the chemicals used were the chemically pure preparations of reliable manufacturers. The water used in making up test solutions was redistilled in glass apparatus. All experiments were made in duplicate, and were repeated when occasion demanded. A fairly constant temperature of 28–29° C. was maintained for the cultures. Beet decoction, made by boiling 450^{gm} fresh weight of sugar beet root in $1{,}000^{cc}$ tap water, was the usual basis of nutrient agar and gelatin media. The stock decoction was diluted two to ten times for use.

TESTS FOR CHEMOTROPISM.

The capillary tube method.—In the first tests PFEFFER'S (**26**) method with capillary tubes was used. These tubes were filled with the chemical solutions under the air-pump, were rapidly rinsed in sterile distilled water, and were placed on the cover glasses in drops of the culture medium, while it was still liquid. The cover glasses were then inverted over Van Tieghem cells made up in the way described by CLARK (**3**), and were sealed to the cell rim with vaseline. A small quantity of liquid, the same in composition as that used in making up the culture medium, was placed in the bottom of each cell. Observations were made when the hyphae were 40–75 spore-diameters in length, and again three or four hours later when growth had become indefinitely great. In estimating the effects of the chemicals, regard was had for the hyphae from spores lying within a radius of one lumen diameter from the opening of the tube, and such other hyphae from more distant spores as entered this area. Only those were held to be chemotropically affected that showed a turning out of their former courses toward or away from the tube opening. In recording the observations MIYOSHI'S method was used; to denote a turning away of 12 per cent. to 37 per cent., the symbol *r* was used; for a turning away of less than 12 per cent. and an attraction of less than 12 per cent., the symbol *o;* for an attraction

of 12 per cent. to 37 per cent., a_1; for an attraction of 37 per cent. to 62 per cent., a_2; etc. An interrogation point indicates a value near the lower limit of the class indicated.

This method was used in the preliminary testing of a large number of representative chemical substances with *Sterigmatocystis nigra* and *Mucor mucedo; Botrytis vulgaris* was used with a few that gave decided effects. The series included the sulfate of sodium, of magnesium, of calcium, of ammonium, of potassium; the normal phosphate of ammonium, of potassium; the dibasic phosphate of ammonium, of potassium; the monobasic phosphate of potassium, of sodium, of calcium; the chlorid of potassium, of ammonium, of calcium, of magnesium, of sodium, of lithium; the nitrate of potassium, of ammonium, of calcium, of magnesium, of sodium; the oxalate of magnesium, of potassium; the tartrate of magnesium, of potassium, of sodium-potassium; the bitartrate of potassium; the lactate of magnesium; the malate of magnesium, of ammonium; the acetate of potassium; acetic, lactic, tartaric, malic, oxalic acids; cane sugar, dextrose, galactose, maltose, lactose, starch; glycerin, ethyl alcohol; active pepsin, boiled pepsin, trypsin, peptone, mannit agaricin, casein, asparagin, urea; meat extract, beet decoction, bean decoction, distilled water; mercuric chlorid, copper sulfate, copper acetate, lead nitrate, zinc nitrate, iron sulfate, iron chlorid, potassium ferrocyanid. In the case of such of these compounds as were tested by MIYOSHI, concentrations were used that would give the maximum effect of attraction or repulsion. With nearly all compounds two, and with many three, concentrations were tested.

The hyphae showed a tendency, in small numbers, to turn toward the tubes in the majority of tests; but in only a few instances were more than about 37 per cent. of the hyphae in the observed area

TABLE I

SPECIES	$CaH_4(PO_4)_2$, 2%	KH_2PO_4, 2%	$MgSO_4$, 2%	$NaNO_3$, 2%	$(NH_4)_2HPO_4$, 2%	NH_4NO_3, 2%	Acetic Acid, 5%	Malic Acid, 5%	Pepsin (active), 2%	Pepsin (boiled), 2%	Trypsin, 1%	Meat Extract, 10%	Ammonium Malate, 2%
Sterigmatocystis nigra	r	...	r	...	...	r	...	...	...	...	...	...	r
Mucor mucedo	...	a_2	...	r	a_2	...	r	a_2	a_2	a_2	a_2	a_2	a_2
Botrytis vulgaris	...	...	...	...	...	...	...	...	a_2	a_2	...	...	...

affected. The repellent effect was much less marked, never affecting more than one-fourth of the hyphae, and even in the most marked cases some of the hyphae would grow toward the tubes and enter them. Table I gives the most marked instances of attraction and repulsion noted in the series; the symbols have been explained above.

In Table II is given a comparison of the effects of a number of representative compounds as observed by MIYOSHI and in the present investigation. Leaving out of consideration *Saprolegnia ferax*, the extreme effects recorded in MIYOSHI's table were obtained

TABLE II

REAGENT	MUCOR MUCEDO		ASPERGILLUS NIGER	STERIGMATOCYSTIS NIGRA
	I	II	III	IV
Monobasic potassium phosphate, 2 %	a_3	a_2	a_1	a_1
Monobasic sodium phosphate, 2 %	a_2	a_2 ?	a_1	a_1
Neutral ammonium phosphate, 2 %	a_4	a_1	a_2	a_1
Neutral ammonium phosphate, 0.2 %	...	a_1	...	a_1
Neutral ammonium phosphate, 0.1 %	a_1	...	a_1	...
Ammonium nitrate, 2 %	a_3	a_2 ?	a_2	r
Potassium nitrate, 2 %	r	a_1	r	o
Calcium nitrate, 2 %	r	a_1	r	o
Magnesium sulfate, 2 %	r	o	r	r
Ammonium chlorid, 3 %	a_3	o	a_1	o
Potassium chlorid, 2 %	r	o	r	o
Sodium chlorid, 2 %	r	o	r	o
Sodium-potassium tartrate, 2 %	r	a_2 ?	r	o
Cane sugar, 20 %	a_1	o	a_1	o
" " 10 %	a_4	...	a_3	...
" " 5 %	...	o	...	o
" " 1 %	a_3	a_1	a_2	a_1 ?
" " 0.1 %	a_1	o	a_1	o
Lactose, 2 %	a_1	...	a_1 ?	...
" 1 %	...	a_1	...	a_1 ?
Maltose, 2 %	a_1	...	o	...
" 1 %	...	o	...	a_1 ?
Dextrose, 10 %	a_4	...	a_2	...
" 5 %	...	o	...	a_1 ?
" 2 %	a_4	...	a_3	...
" 1 %	a_3	r ?	a_1	o
Ethyl alcohol, 1 %	r	a_2 ?	r	a_2 ?
Glycerin, 10 %	a_1	o	a_1 ?	a_1 ?
Asparagin, 2 %	a_2	a_2 ?	a_1	a_1 ?
Urea, 2 %	o	a_1 ?	o	a_1 ?
Peptone, 2 %	...	o	...	a_1 ?
" 1 %	a_3	...	a_2	...
Meat extract, 10 %	a_4	a_2	a_4	...
" " 1 %	a_3	a_1	a_2	a_1
" " 0.1 %	a_1	o	a_1	a_1 ?
" " 0.01 %	a_1	o	o	a_2 ?

with *Mucor mucedo* and *Aspergillus niger;* the values given by him for these forms are to be found in columns I and III below. It was impracticable to use *Asperillgus niger* in the present study, and *Sterigmatocystis nigra* was substituted with the understanding that the two forms are not always distinguished. The present results for *Mucor mucedo* and *Sterigmatocystis nigra* are given below in columns II and IV respectively.

In the control cultures, where distilled water was used in the tubes, the effect was the same as for the majority of chemical substances; that is, 10–30 per cent. of the hyphae turned toward the tubes. The same amount of positive turning was observed in the case of all the strongly toxic compounds used. Even with a 0.05 per cent. solution of mercuric chlorid and a 1 per cent. solution of copper sulfate, which completely inhibited germination within a radius of eight to twelve tube diameters from the openings, the hyphae not only grew across the diffusion areas, but 10–30 per cent. of those approaching the openings turned toward them and grew for a considerable distance into the tubes.

Although four concentrations of cane sugar ranging from 20 to 0.1 per cent. and four concentrations of meat extract ranging from 10–0.01 per cent. were used, no definite relation between the strength of stimulus and that of response was apparent.

Two corresponding series were made with ten representative compounds; in one series sugar-beet agar was the culture medium; in the other, distilled-water agar. No difference in the behavior of the hyphae due to a difference in the media in which they grew could be observed.

Tests with mica plates.—Thin sheets of mica were cut into pieces about 25×16^{mm}; these were perforated with a needle, the holes being $0.1–0.15^{mm}$ in diameter, and about 2^{mm} apart. Covers of suitable size were cut from glass 1^{mm} thick. A layer of gelatin or agar was placed on a cover, a mica plate was placed on this just before it hardened, and a second layer was placed above the plate. The chemical to be tested was made up in double strength solution in redistilled water, and one volume of this was added to one volume of gelatin or agar, also made up double strength in redistilled water. It was usually found convenient to have the layer containing the

chemical next to the cover, the spores being distributed in the outer layer. The cover was inverted over a stender dish of 36^{mm} diameter containing distilled water, and was sealed with a coating of vaseline around the rim of the vessel.

This method seemed to possess distinct advantages over the one with capillary tubes: the numerous perforations made it possible to make a large number of observations from a single preparation;

TABLE III

	I. Monilia fructigena	II. Monilia sitophila	III. Sterigmatocystis nigra	IV. Botrytis vulgaris	V. Cercospora apii	VI. Sphaeropsis malorum
Neutral potassium phosphate, 2 %	12	19	24	22	33	22
" " " 0.2 %	21	20	18	25	36	25
Monobasic potassium phosphate, 2 %	29	22	24	26	36	23
" " " 0.2 %	20	26	23	20	25	21
Dibasic ammonium phosphate, 2 %	13	20	21	20	15	23
Calcium phosphate, 0.2 %	49	41	34	31	35	29
Phosphoric acid, 0.2 %	48	30	35	39	23	30
Malic acid, 1 %	×	×	24	×	38	×
" " 0.1 %	21	21	26	21	17	×
Tartaric acid, 1 %	×	20	43	22	19	18
" " 0.1 %	25	27	34	25	24	22
Oxalic acid, 1 %	×	17	23	×	17	×
" " 0.1 %	23	22	23	34	11	40
Cane sugar, 5 %	38	32	24	31	20	48
" " 0.5 %	30	37	20	30	20	55
" " 0.05 %	30	35	24	33	23	42
Glucose, 5 %	39	39	20	34	30	37
" 0.5 %	40	24	23	39	20	30
Peptone, 2 %	14	28	21	13	20	19
" 0.2 %	26	23	20	21	13	22
Pepsin (active), 2 %	24	20	19	42	23	30
" " 0.2 %	25	30	22	27	31	22
Pepsin (boiled), 2 %	26	31	36	33	40	37
Asparagin, 0.1 %	19	23	30	23	30	15
" 0.1 %	15	23	30	20	30	24
Copper sulfate, 0.005 %	29	21	21	25	34	31
Dibasic sodium phosphate, 2 %	35	25	26	26	15	20
" " " 0.2 %	38	30	27	20	26	23
Starch paste, 0.4 %	34	24	20	21	27	25
" " 0.4 %	44	27	22	20	26	15
Maltose, 2 %	33	30	22	21	25	14
" 0.2 %	36	29	28	31	30	28
Control (distilled-water gelatin)	28	30	29	24	22	18

NOTE: The symbol × indicates that germination was inhibited. *Uromyces caryophyllinus* was tested with the majority of these substances, in the few instances in which growth was sufficiently great for a determination, the turning was practically the same as for the other fungi.

the medium containing the chemical and that containing the spores could be more nearly equalized in amount and in consistency; fewer hyphae would take a course that would lead them through the openings without apparent turning; better sterilization could be secured; and there was less difficulty in making up the preparations.

In making the counts, hyphae within a radius of one opening diameter from the margin of each opening were considered; the hyphae within such an area were classed in the counts as those turning toward the openings, those turning away from the openings, and those apparently indifferent. After an examination of the entire preparation in each case, those holes were selected for the counts which represented the average condition. In calculating the percentages from the counts, the difference between those attracted and those repelled was made the dividend, and the total number within the observed area was made the divisor. The results are shown in Table III.

If the percentages of turning toward distilled-water gelatin as determined by the control experiments, be deducted from the percentages of turning toward the various chemical compounds, it will be found that in only three instances would the difference, which would be supposed to indicate the amount of turning due to chemical influences, approximate 25 per cent., or about the average of the lowest of the several degrees of positive chemotropism recognized by MIYOSHI. Most of the values, even for supposedly highly attractive substances, are very near the controls.

Evidently, the results thus far have not been favorable to the theory of chemotropism. But it was thought that the fungi, all of which grew perfectly normally in gelatin made up in distilled water, might turn more strongly from some other medium in which there was less of available nutriment, to one having an abundance. In agar made up with distilled water, the fungi germinated after a greater length of time and grew more slowly; but the turning from this non-nutrient agar toward a nutrient salt solution favorable for fungous growth was no more marked than from agar containing the same proportions of the nutrient salts to the nutrient salt solution; nor did the nutrient solution seem to attract from either medium more strongly than did distilled water. This test was made with

Sterigmatocystis nigra, *Mucor mucedo*, and *Mucor stolonifer* by the capillary tube method.

It is possible, although there is evidence against it in some of the previous experiments, that the diffusion of the solutes might be rapid enough to bring about practical uniformity in the media before the germination of the spores. To determine whether the time at which the stimulus is applied has an influence upon the reaction, series were made up with two of the more slowly growing species, *Botrytis vulgaris* and *Monilia fructigena*, the spores being distributed in non-nutrient gelatin placed above the mica plates. Four duplicate series were arranged. In one the layers of gelatin containing the substances to be tested were placed below the plates at the time of making up the cultures; in another series these layers were added just as the spores began to germinate; in a third, after the most vigorous hyphae had attained a length of 15 to 20 spore-diameters; in a fourth when the length equaled 40 to 50 spore-diameters. The final counts were made a little later, when the growth was about 75 spore-diameters. The results are given in Table IV for the five compounds tested and the control. The numbers in columns I indicate the average number of hyphae in the area around each hole; those in columns II, the percentages of turning toward the holes.

TABLE IV

Species	Hours Until Application	Dextrose 4.5%		$CuSO_4$ 0.01%		Oxalic acid 0.05%		KH_2PO_4 2%		$(NH_4)NO_3$ 2%		Non-nutrient gelatin	
		I	II	I	II	I	II	I	II	I	II	I	II
Botrytis vulgaris	0	11	35	11	38	6.3	34	4.0	37	5.5	36	9.2	34
	10	9.0	35	6.0	30	7.8	36	5.4	30	7.2	36	7.6	34
	13	11	33	5.5	34	6.4	25	6.4	31	11	33	7.2	35
	16	3.3	30	7.6	34	5.5	30	5.8	35	5.0	32	5.6	36
Monilia fructigena	0	9.3	38	10	30	11	28	3.7	31	8.2	34	4.4	26
	10	9.5	36	11	30	11	30	3.7	28	6.8	25	11	32
	15	13	33	7.5	27	10	32	4.3	30	5.4	27	13	27
	20	17	32	8.7	27	9.3	29	5.3	29	5.1	26	8.3	30

It is evident that the time of application has little or no effect upon the amount of turning. It will also be seen by comparing these

results with former ones, that it is immaterial, as far as the percentage of turning is concerned, whether the spore-containing layer is above or below the one containing the test substance.

Other culture media were used, such as 10 per cent. and 25 per cent. glycerin, gelatin made up to contain 10 per cent. of glycerin and to contain 5 per cent. of cane sugar. With none of these were there more distinct indications of chemotropism than in former tests, in which non-nutrient gelatin was the culture medium. This would indicate that the available nutriment and the concentration of the medium have no effect.

The final test along this line was made with silica jelly, a medium free from organic matter and of suitable consistency. The method of preparation was that used by MOORE (**23**), except that dialysis was accomplished satisfactorily with parchment paper. In order to secure the proper coagulation of the medium, it was necessary to add mineral salts to all media used. A nutrient salt solution, containing 1^{gm} of ammonium nitrate, 0.5^{gm} of monobasic potassium phosphate, 0.25^{gm} of magnesium sulfate, a trace of ferric chlorid, and 5^{gm} of glucose, in a volume of 100^{cc}, was made the basis of the work. It would seem that if fungi show chemotropism under any conditions it would be by turning from a medium lacking some one or more of the elements necessary for full development, toward the diffusion centers of compounds supplying the missing element or elements, or toward a full nutrient solution.

In the tests each compound in turn, except ferric chlorid, was omitted from the silica jelly containing the spores, and in each case jelly containing the omitted compound in proper proportion on the one hand, and full nutrient jelly on the other were used on the opposite side of the mica plate from the above-mentioned spore-containing layer. Control tests were carried on with jelly lacking one and the same compound on each side of the plate, and also with full nutrient jelly on each side of the plate. To reduce evaporation, the lower layer was covered with an unperforated mica plate. The results are given in Table V, in percentage of response by turning from the first-named medium in which the spores were sown to the contrasted one.

There is a very striking uniformity in the percentages, and this

TABLE V

Contrasted Cultures in Silica Jelly	Mucor stolonifer	Sterigmatocystis nigra	Monilia sitophila	Botrytis vulgaris	Monilia fructigena
Full nutrient : full nutrient	35	32	32	33	29
Lacking glucose : lacking glucose	32	36	37	30	33
Lacking glucose : containing glucose	27	30	34	30	26
Lacking glucose : full nutrient	30	36	33	30	30
Lacking KH_2PO_4 : lacking KH_2PO_4	33	30	29	33	26
Lacking KH_2PO_4 : containing KH_2PO_4	36	36	31	30	28
Lacking KH_2PO_4 : full nutrient	32	30	33	36	28
Lacking $(NH_4)NO_3$: lacking $(NH_4)NO_3$	35	35	33	36	28
Lacking $(NH_4)NO_3$: containing $(NH_4)NO_3$	34	32	32	33	29
Lacking $(NH_4)NO_3$: full nutrient	34	33	33	31	32
Lacking $MgSO_4$: lacking $MgSO_4$	33	31	33	33	27
Lacking $MgSO_4$: containing $MgSO_4$	39	34	33	33	29
Lacking $MgSO_4$: full nutrient	29	33	32	35	30

under conditions that would be presumed to be most favorable for chemotropic reaction.

Tests with epidermis and celloidin films.—To test the effect of physically different perforated sheets as well as effectually to repeat the methods used by former investigators, use was made of celloidin films which had been perforated, and of strips of epidermis of *Yucca aloifolia*. This gave, with reference to physical properties, a range from the wholly impermeable mica plates on the one hand to the semipermeable celloidin films on the other.

The tests with epidermis were made with *Monilia fructigena*, *Sterigmatocystis nigra*, *Botrytis vulgaris*, *Sphaeropsis malorum*, and *Mucor stolonifer*, the spores of which were distributed in non-nutrient 8 per cent. gelatin above the epidermis in its final position; gelatin layers containing 5 per cent. cane sugar, 4.5 per cent. dextrose, 0.01 per cent. copper sulfate, 0.1 per cent. oxalic acid, 0.2 per cent. phosphoric acid, and non-nutrient gelatin, were spread below the epidermis. Under these conditions the penetration of the stomates or turning toward the stomates was practically zero. When no culture medium was used, the spores being merely spread on the under surface of the epidermis, hyphal growth was good. A few hyphae of each species grew through the stomates; but there was no evident turning toward them, and in no case was there penetration of more than one or two per cent. of the stomates.

In similar series with celloidin films, the turning from one gelatin layer to another was about equal to that obtained with mica plates. When the spores were spread on the film without a culture medium, very few of the hyphae grew through the holes, the percentage of turning being negligible. The hyphae in these cases were surrounded by a distinct film of condensed moisture.

TESTS FOR OTHER FACTORS.

The tests thus far have failed to give evidence of the existence of any marked chemotropism. There has been at the same time a considerable and fairly constant turning of the hyphae from a medium containing spores to a sterile medium, when these were separated the one from the other by any one of several partitions. Since this turning is apparently unaffected by the chemical relationships of the media employed, the cause of the turning must be sought in other factors. Two possibilities at first present themselves; the mechanical partitions may have a thigmotropic or other influence, or the germinating spores themselves may affect the direction of growth.

Tests without mechanical partitions.—A slight modification of the method employed by CLARK (4) was used. A large drop of agar, 8^{mm} in diameter, was placed in the center of a sufficiently large square of glass; this drop was surrounded by four drops of about 5^{mm} diameter, equidistant from the first, and with a space of about 3^{mm} between each smaller drop and the larger one. Non-nutrient agar and 10 per cent. sugar-beet agar were used for the drops, and were arranged in four combinations: the central drop was of nutrient agar and two small drops diagonally opposite each other were of nutrient agar, the other two being non-nutrient; the central drop was of nutrient agar, two small drops adjacent to each other of nutrient agar, and the other two of non-nutrient agar; two similarly arranged combinations had central drops of non-nutrient agar. The fungi used were *Monilia sitophila*, *Mucor stolonifer*, and *Botrytis vulgaris*. A few spores were sown with the platinum needle at the center of the large drop in each preparation; the cover was inverted over a stender dish containing distilled water and was sealed to its rim. The growth was watched until the hyphae had grown about two-thirds of the distance from the center to the margin

of a large drop; the preparations were then opened, and with a sterile needle the small drops were pushed up until their edges came in contact with the larger drops. Later observations showed that the hyphae of the three fungi grew readily from either medium into a similar or a dissimilar medium, and with the same percentage of turning. An equal amount of turning toward the agar drops was observed in the case of those hyphae which had grown beyond the bounds of the larger drops on the moist glass; whether the agar was nutrient or non-nutrient seemed immaterial. The turning was apparent at a considerable distance from the surface film in so large a percentage of cases as to negative the supposition that the physical condition of the film has an influence.

A further test without mechanical separation was made by placing small crystals of cane sugar, copper sulfate, oxalic acid, monobasic potassium phosphate, and ammonium nitrate in the center of layers of non-nutrient gelatin on cover glasses. Spores of *Monilia fructigena*, *Botrytis vulgaris*, *Sterigmatocystis nigra*, *Mucor stolonifer*, and *Monilia sitophila* were used; in some instances they were evenly distributed in the gelatin, in other instances the gelatin was inoculated by being touched with the needle at several points varying in distance from the crystal. In no case was there any distinct turning toward or away from the diffusion center.

The effect of hyphae upon the direction of growth.—Clark (4) explains his results by supposing that the fungus secretes some substance to which the growing hyphae are negatively chemotropic. While this hypothesis would very well explain his results, he seems not to have made it the subject of experimental study.

It may be reasonably assumed that if a fungus is negatively chemotropic to its own secretion, the stimulus to turn away from an area containing the fungus would, in early stages of growth, be in some degree proportional to the amount of mycelium in that area.

To determine whether the amount of mycelium has an effect, inoculations were made with differing numbers of spores; the growth was from non-nutrient gelatin and gelatin containing M/4 solution of dextrose, and was toward similar as well as different media as indicated in Table VI, where the results are given. The direction of growth is from the first-named medium to the second. Columns

I give the average number of spores per hole, and columns II give the percentages of turning toward the holes.

TABLE VI

Contrasted Cultures in Gelatin	Sterigmatocystis nigra		Monilia sitophila		Botrytis vulgaris		Monilia fructigena		Mucor stolonifer	
	I	II	I	II	I	II	I	II	I	II
Non-nutrient : M/4 dextrose	4.1	34	5.0	24	6.8	27	5.0	20	15	31
	15	32	16	25	22	37	7.3	26	54	36
M/4 dextrose : M/4 dextrose	8.5	24	4.2	25	8.3	29	4.8	26	5.0	25
	22	36	23	38	23	37	21	36	20	38
M/4 dextrose : non-nutrient	8.0	25	7.7	26	6.7	28	6.0	28	6.3	31
	19	35	17	36	23	37	18	29	14	31
Non-nutrient : non-nutrient	3.0	33	5.0	30	8.0	31	4.5	22	7.1	28
	21	40	16	40	18	39	17	34	36	41
M/4 glycerin : M/4 dextrose	3.9	30	4.3	27	6.3	20	7.0	28	17	29
	22	38	19	39	18	36	18	34	31	35
Non-nutrient : 0.01% $CuSO_4$	3.1	24	5.3	28	6.3	28	5.0	27	18	33
	20	39	15	39	21	39	16	32	43	39

There is indication that the number of hyphae in a given area and the amount of turning from that area are correlated. It may be said of this, as of succeeding tests, that it is at best only relative. It is manifestly impossible to eliminate growing hyphae from the experiments, and their effect is the very factor to be tested.

A test was made by comparing preparations in which only one layer of gelatin was inoculated, with preparations in which both layers were inoculated. Two parallel series were prepared: in one the spores in the lower layer were about twice as numerous as in the other series. An examination of Table VII, which gives the results, shows that the percentage of turning toward a layer containing hyphae is less than toward a sterile layer; and in either case the abundance of mycelium in the layer influences directly the turning

TABLE VII

Monilia sitophila			Sterigmatocystis nigra			Mucor stolonifer			Mucor mucedo			Phycomyces nitens		
I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
6.5	0	26	6.5	0	32	5.5	0	32	11	0	29	7	0	28
12	0	35	15	0	38	14	0	39	23	0	34	14	0	37
4.5	7	20	5.0	8	23	4.5	7	15	11	19	25	9	16	24
9	6	22	12	8	24	9.5	7	24	19	11	30	16	9	29

of the hyphae from that layer. Column I gives the average number of hyphae per hole in the layer *from* which the turning takes place; column II, the average number of hyphae per hole in the layer *toward* which turning takes place; column III, the percentage of turning.

To determine whether this negative turning is due largely to chemical changes in the culture medium, beet decoction in which a fungus had grown was tested with that fungus. The sugar-beet decoction was diluted to one-third the strength of the stock solution. When a good amount of mycelium had been formed, the liquid was filtered under sterile conditions at room temperature. The filtrate was replaced in the incubator in the case of those species which had fruited, and was allowed to remain for twenty-four hours in order that any spores that had passed through the filter might germinate; it was then refiltered. In this way a practically sterile medium was obtained. One volume of this decoction was added to one volume of double-strength gelatin, and tests were made with this

TABLE VIII

		A		B	
		Growth toward Gelatin Made Up with Beet Decoction in Which Fungus Had Grown		Growth toward Gelatin Made Up with Fresh Beet Decoction. Control	
		I	II	I	II
I.	Botrytis vulgaris	6.2	29	5.3	33
		15	31	15	35
II.	Sterigmatocystis nigra	10	29	9.0	34
		19	27	17	37
III.	Penicillium glaucum	8.0	23	12	31
		18	22	28	23
IV.	Monilia sitophila	8.0	22	9.0	26
		23	25	20	33
V.	Mucor stolonifer	19	32	22	38
VI.	Agaricus fabaceus	49	24	50	32
VII.	Coprinus micaceus	31	24	43	30
VIII.	Daedalia quercina	46	26	35	31

in comparison with control beet decoction in which there had been no fungous growth. The results are shown in Table VIII. Column I gives the average number of hyphae per hole; column II, the percentage of turning toward the holes.

The percentages for all of the fungi in this table, except *Mucor*

stolonifer, have been computed from four distinct series. In this way erratic results have been nullified, and the difference in effect, although not marked, may be regarded as constant. There is certainly a lessened attractive influence in the case of the decoction in which the fungi have grown; this might be due to the mere abstraction from the decoction of nutrient substances, or to the conversion of compounds occurring in the decoction into compounds which are repellent in their effect, or to the secretion of products by the fungus which have a repellent effect. That the first is probably not the case is to be inferred from previous tests, in which these fungi have been found to grow as readily toward distilled water and other non-nutrients as toward nutrients; a mere decrease, therefore, in the amount of available nutriment could hardly have a pronounced effect. We must conclude, therefore, that a medium in which a fungus has grown may become less attractive, or more repellent, to the fungus through the agency of some undetermined substance or substances, which are secreted or otherwise formed by the growing fungus; this reaction would be a special kind of negative chemotropism.

Miss FERGUSON (**13**) found that germinating spores of *Agaricus campestris*, or bits of older growing mycelia, have a very marked effect in causing the germination of spores of this species; at the same time there seems to be a retarding effect upon the growth of the protruded germ-mycelium. Mycelium that is not growing, masses of ungerminated spores, or growing mycelia of other fungi do not have the same influence over germination. Her observations lead her to suppose that oxygen or carbon dioxid is not the determining factor, but that some secretion is formed which stimulates or makes possible the emission of the germ-tubes. Other observations relative to the influence of germinating spores upon the growth of fungi have been made by KIHLMAN (**16**) and REINHARDT (**28**); to these reference has already been made.

Numerous instances have been recorded of the influence by various plant cells upon the direction of movement and of growth of other cells of the same or a different kind, and the general terms cytotaxis and cytotropism have been applied to this peculiar sort of chemical influence. In the cases enumerated by PFEFFER (**27**,

sec. 155), the effects seem to be due either to the excretion of a hypothetical specific substance which furnishes the stimulus, or to changes in the relative proportions of oxygen and carbon dioxid through respiratory or photosynthetic activities. Since these phenomena seem to be analogous in a general way to the above-described turning of fungous hyphae, and since the term cytotropism indicates nothing as to the exact nature of the ultimate stimulus-substance, this term might be found a convenient one for designating the present cases.

The effect of moisture.—The circumstance that a number of preparations in which the culture medium had become evidently dry, gave large percentages of turning, suggested that the hyphae might react to a hydrotropic stimulus. Layers of agar containing spores of several fungi were spread on cover glasses, and sterile strips of filter paper were placed in contact with the agar and allowed to dip into the water of the Van Tieghem cell. The average percentage of turning toward the strips for those spores within a distance of 0.35^{mm} from their edges was 40 per cent. for *Penicillium glaucum;* 50 per cent. for *Mucor stolonifer, Monilia sitophila,* and *Sterigmatocystis nigra;* 55 per cent. for *Mucor mucedo;* 57 per cent. for *Botrytis vulgaris;* and 60 per cent. for *Monilia fructigena* and *Sphaeropsis malorum.*

The percentages given are less than they would be if a smaller area about each strip had been considered; this may be due in part to the circumstance that the spores were rather thickly sown, and the hyphae from those nearest the strip, being numerous, exerted an effective repellent influence on those more distant, causing them to grow away from the strip. Notwithstanding this, the evidence of positive hydrotropism for these fungi was quite conclusive.

As a further test, mica plates were cut to fit Van Tieghem cells and were perforated; a drop of non-nutrient gelatin was placed on each and covered with a perforated plate small enough to fit inside the cell; upon this was placed another layer of non-nutrient jelly containing the spores; another perforated plate was added, and a third layer of gelatin, sterile like the first; the lower surface of this was left uncovered. The mica cover was inverted over a cell containing water, during the time required for the proper growth of the fungi, evaporation took place from the now uppermost layer to

the surrounding atmosphere, as was apparent from the dry condition of the gelatin around the holes in the uppermost plate; water diffused from the lower layers to supply the deficiency. In this way it came about that the middle layer, which contained the spores, was moister than the uppermost layer, but drier than the lowermost. There was observed a very decided turning of the hyphae toward and through the openings in the third plate, which separated the middle and lowermost layers, while comparatively few grew toward the uppermost layer. The estimated percentages are shown in Table IX.

TABLE IX

Directions	Monilia fructigena	Monilia sitophila	Sterigmatocystis nigra	Botrytis vulgaris	Penicillium glaucum	Sphaeropsis malorum	Mucor mucedo	Mucor stolonifer	Phycomyces nitens
From middle layer to lowermost layer..	43	70	80	55	68	65	73	63	65
From middle layer to uppermost layer..	20	20	20	30	18	15	18	15	18

Experiments were set up in which very firm gelatin (16 per cent.) containing the spores was covered with mica plates having a few perforations. The plates were sealed to the covers by an application of vaseline around their margins. The covers were then inverted over stender dishes level full of sterile distilled water. In this way the water came in contact with the gelatin only through the perforations, and diffused from these through the gelatin layer. Hyphae of *Mucor stolonifer* grown under these conditions showed a tendency to grow toward the openings from a distance of 1.5^{mm}, but on coming within 0.5^{mm} of the openings, the course was changed, and the hyphae circled the openings in lines more or less concentric with their margins. The majority of those nearer the openings than 0.5^{mm} grew in a radial direction away from them. In a few instances hyphae grew into the water. *Mucor mucedo* showed a quite decided turning toward the holes, and about 65 per cent of the hyphae within a radius of three hole-diameters turned through them and grew into the water. With *Botrytis vulgaris* about 40 per cent., and with *Penicillium glaucum* 85 per cent. of the hyphae within a corresponding area were affected positively. In every case the growth

in the water was in all directions, directly downward, as well as radially in a horizontal plane. The value of the control cultures, which were duplicates in all respects excepting that the dishes were only partly filled with water, was vitiated by the accumulation of condensed moisture in comparatively large drops about the openings in the plates. This caused an unmistakable turning toward the holes, which was not so decided, however, as in the test cultures.

It is evident from these results that all of the fungi tested in this regard are, under the conditions of experiment, positively hydrotropic; but *Mucor stolonifer* may under certain conditions show a negative hydrotropism. This response to a hydrotropic stimulus probably accounts in large measure for the constant turning toward protected layers from those more exposed, which latter may have become drier through evaporation.

A sharp distinction between hydrotropism and rheotropism on the one hand, or between hydrotropism and osmotropism on the other, cannot in all cases be made, although these phenomena in typical cases are quite distinct. The phenomena here reported are probably due primarily to differences in the moisture content of the layers, and not to water currents, either molar or molecular. For this reason the term hydrotropism has been applied, which is not in agreement, however, with the current view (PFEFFER, **27**, p. 592), that in the case of the fungous mycelia heretofore studied, osmotropism and rheotropism, but not hydrotropism, have been established. It is further recognized that chemical rather than other properties of water furnish the effective stimulus, in which event hydrotropism would properly be regarded as a special kind of chemotropism.

Aerotropism.—Under the conditions prevailing in some of the experiments above described, there was doubtless an inadequate supply of oxygen, as when a medium poor in oxygen was enclosed between impervious plates. There was then a very decided tendency for the hyphae to turn toward the edges of the plates. The observation of this phenomenon from time to time suggested that the fungi might show an aerotropic sensibility, either as a positive reaction to oxygen, or as a negative reaction to carbon dioxid.

In order definitely to test the matter, experiments were arranged

in which the growth toward holes in mica plates could be observed when the plates separated normal non-nutrient gelatin from non-nutrient gelatin saturated with carbon dioxid on the one hand, and from normal non-nutrient gelatin on the other. The fungi used were *Penicillium glaucum*, *Sterigmatocystis nigra*, *Mucor mucedo*, *Botrytis vulgaris*, *Monilia fructigena*, *Monilia sitophila*, and *Phycomyces nitens*. In no case was the percentage of turning toward the carbon dioxid gelatin greatly different from that toward the control gelatin.

As a further test, a layer of gelatin containing spores was placed below a perforated mica cover for a Van Tieghem cell, and a perforated mica plate, small enough to fit inside the cell, was placed below the gelatin. A layer of normal gelatin was spread below this last plate, and a layer of carbon dioxid gelatin above the cover. This preparation was sealed to the cell rim, and the whole placed under a bell jar practically filled with carbon dioxid and kept at a room temperature of 21–24° C. Efforts were made to have the moisture conditions equal within and without the cells; and the exposed gelatin layers, which served very well as indicators, showed no difference in this respect until after the observations on the majority of the preparations had been made, although there was drying of the outer gelatin layer by the time the more slowly growing fungi had reached the proper stage. The same fungi were used in this experiment as in the preceding one, with the addition of *Mucor stolonifer*. In most instances the turning toward the gelatin containing carbon dioxid and exposed to an atmosphere of carbon dioxid, was as great as toward normal gelatin; the growth, however, was less vigorous in the former case. In those preparations in which there was less turning toward the carbon dioxid gelatin, this gelatin had become evidently rather dry.

It is to be concluded, therefore, that the observed turning toward the edges of preparations is not due primarily to aerotropic sensibility. The experiments also negative the supposition that the observed repellent influence of growing hyphae may be due to the consumption of oxygen or to the production of carbon dioxid by the fungus, or to both.

Osmotropism.—Notwithstanding the fact that osmotropism is

intimately associated with chemotropism, and that many of the tests for the latter are in equal measure tests for the former, direct tests were made by growing the fungi in media of higher osmotic pressure and of lower osmotic pressure than the test media, as well as in an isosmotic medium; glycerin, a good nutrient substance, and yet a substance reported by MIYOSHI to be neutral in its chemotropic effect, was used to give the desired concentration to the culture media. The series failed to show that the concentration of the culture medium has an effect upon the amount of turning. However, no excessively high concentrations of mineral salts were used.

Other tropic phenomena.—Under conditions that would favor a manifestation of geotropism and of thigmotropism, there was no indication that these are concerned in determining the direction of growth of the fungi. The effects of light and of heat were in no way tested, but they probably do not enter as factors.

Biological significance.—The conclusions reached in these studies may be found to have a somewhat important bearing upon the biological problem of infection by parasitic fungi. In the absence of any experimental investigation, nothing definite can now be said. It would seem, however, that the drying of dew and other surface moisture in which spores had gerimnated, might be a condition favoring the hydrotropic turning of the germ-tubes toward the stomates, especially if the cells within are over-distended with water, which has frequently been observed to be a condition favorable for infection; if the germ-tubes are numerous in the vicinity of a stomate, the repellent influence of these upon one another would cause some to seek the unoccupied region within the stomate. At all events the phenomenon of the entrance of germ-tubes, whether by way of the stoma or through the cuticle, is a complex one, of which many factors remain undetermined. That mere entrance is probably not due to specific peculiarities, either of host or parasite, is evidenced by the recent work of Miss GIBSON which has been mentioned by MARSHALL WARD (**33**). Miss GIBSON found that spores of various members of the Uredineae sent their germ-tubes readily into the stomates of plants widely different from their hosts, and which they were unable to infect. MARSHALL WARD met with numerous instances of the same phenomenon. This would indicate

that the entrance of a hypha into a stoma is merely a preliminary act, distinct from infection proper, and controlled by general conditions, while the fate of the hypha after its entrance is determined by complex reactions between parasite and host, which are largely specific in their nature. In the light, then, of known facts, no simple explanation, such as the theory of chemotropism due to the presence of specific chemical compounds, is adequate. Chemotropism may possibly be one factor in the complex phenomenon, but it is certainly not the predominant factor.

CONCLUSIONS.

Various tests upon a number of fungi failed to indicate the existence of any definite chemotropic sensibility to nutrient substances or other chemical compounds in solution. If positive chemotropism exists, it is less prominent than other tropic phenomena involved, and was obscured by them.

Those substances which furnished nutriment to the fungi caused a decided growth, often with thickening of the hyphae and an increased branching; but they did not cause a more marked turning of the hyphae toward the diffusion centers than did non-nutrient and toxic substances.

All of the fungi tested show a tendency to turn from a region in which hyphae of the same kind are growing toward one destitute of hyphae, or in which the hyphae are less abundant. The turning toward a medium in which mycelium has grown, but from which the mycelium has been removed, is less marked than that toward a medium in which no mycelium has grown. This may be regarded as a negative reaction to stimuli from chemical substances, which owe their origin in some way to the growing fungus.

Various fungi show a positive hydrotropism; but an overabundance of moisture may cause a negative reaction in certain fungi.

The changing of the direction of growth of fungous hyphae is a complex phenomenon in which at least two factors, cytotropism and hydrotropism, are concerned. Since the complete elimination of neither of these factors is possible, all tests must be relative, and to that extent unsatisfactory.

It would seem that the reactions of mycelium to various stimuli

are not necessarily the same as the reactions, under similar conditions, of sporangiophores, gametophores, and other specialized parts.

The writer wishes to acknowledge his indebtedness to Dr. B. M. DUGGAR for his very helpful suggestion and cirticism, and to Dr. WILLIAM TRELEASE for the opportunity to consult the library of the Missouri Botanical Gardens.

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THE EMBRYOLOGY AND DEVELOPMENT OF RICCIA LUTESCENS AND RICCIA CRYSTALLINA.[1]

CHARLES E. LEWIS.

(WITH PLATES V–IX)

IN June 1903, while collecting liverworts in the vicinity of Ithaca, N. Y., an abundance of material of *Riccia lutescens* was found growing around the edges of dried-up ponds. In some cases the plants formed beautiful rosettes, but usually they grew in irregular clusters, often being so closely crowded together as to cover the ground for several square centimeters.

The individual plants vary greatly in shape and size. The younger light green plants consist of a narrow, thin, ribbon-shaped thallus which has a longitudinal median groove. In the older plants the fore part of the thallus is thickened, very large air cavities being formed. The thallus is attached to the soil by numerous rhizoids from the older part, the apical end being free. On the under side are numerous colorless lamellae.

As the fruiting plant is unknown there is doubt as to the relationship of this species, authorities differing widely as to its status. LINDBERG (**21**) claimed that it was merely a sterile terrestrial form of *Ricciocarpus natans*. UNDERWOOD (**30**) says of it: "approaches certain terrestrial forms of *Ricciocarpus natans*, and possibly derived from that species, but better kept distinct." STEPHANI (**28**) states that it is probably not a Riccia but a sterile marchantiaceous hepatic.

For the purpose of determining the true relationship of the species, Professor ATKINSON suggested the desirability of following the development of the plant through the summer and autumn, and of securing fruiting specimens if possible. He had found young antheridia in plants collected several years before, but had not traced the development further. It also seemed desirable to study the embryology and cytology of the plant if material could be obtained, because comparatively little has been done on these phases of the life history of Riccia.

[1] Contribution no. 106, from the Department of Botany, Cornell University.

BISCHOFF (2) investigated a number of species and settled beyond a doubt the function of the sexual organs. His work was followed the next year by LINDENBERG'S monograph (20) which added little that was new.

The study of the development of Riccia really begins with HOFMEISTER (15), who gave an account of the development of the thallus, sexual organs, and fruit of *Riccia glauca*.

KNY (19) made a careful study of the apical cells and the method of growth of the thallus. He did not secure plants developing from spores but used delicate thalli which had grown crowded together and did not bear sexual organs in the younger parts, so that the regular order of cells was not disturbed. He discovered the origin and manner of growth of the ventral scales and described the development of the sexual organs. Although HOFMEISTER believed that young antheridia and archegonia could not be distinguished, KNY points out that they are distinct after the first walls are formed.

LEITGEB (22) gives a complete account of the method of growth of the thallus in the Ricciaceae. His study of the sexual organs and fruit was in many cases incomplete on account of insufficient material.

BIOLOGY OF RICCIA LUTESCENS.

The account of the biology of *Riccia lutescens* given here is based on field observations extending through two years, together with experiments and observations upon plants kept growing under favorable conditions in the greenhouse and laboratory.

The first observations were made late in June. At that time the plants were growing upon the mud around the edges of ponds. Some of the thalli were very small and delicate, appearing merely as green specks on the mud, while others, which seemed to be older, had the ribbon-shaped form and thickened apical end already described (*figs. 1–3*).

Material was collected and examined from time to time during the summer and autumn, with the expectation of finding plants bearing the sexual organs, because the statement is usually made that the species of Riccia fruit in summer and autumn when growing on the soil. The plants continued to grow well vegetatively throughout the summer, when they were in such a location that they were

supplied with sufficient moisture. In some cases the mud became so dry and hard that the plants were killed, but whenever they were sheltered by a stone or other object, or were growing on the sides of holes, such as cattle tracks, they grew well.

In October all but the youngest and most crowded plants showed the typical *Riccia lutescens* form. At this time young antheridia were found. Material was now collected and fixed from time to time for the purpose of studying the development of the sexual organs. In very few cases were archegonia found in plants collected in autumn. A few young stages were found in plants collected late in November, at which time the older antheridia were almost mature. No further development took place out of doors until spring, because the plants became covered with snow, or with water by the filling up of the ponds, and remained so until April. A quantity of the plants were kept growing on the soil in the greenhouse through the winter, and developed mature sexual organs long before spring. Plants taken from under water in March, just as the ice was going out of the ponds, showed exactly the same form as in November, and little or no further development had taken place. So it seems that the development depends to some extent on temperature, and might be expected to vary with different conditions of climate. A warm winter, in which some growth might take place, would in all probability hasten the development of the sexual organs. Another point of interest is that the submerged plants did not seem to have been injured.

A quantity of material still attached to the soil was taken from under water late in March, and was kept growing in shallow pans in the laboratory so that it could be kept supplied with a sufficient quantity of water for growth but not enough to flood the plants. This was done in order to determine whether the plants would continue the development of sexual organs and fruit in the same way when supplied with a limited amount of water and growing on the soil, as when supplied with a large amount of water which would tend to cause them to break loose and float. It was found that the plants growing on the soil did produce fruit abundantly and at the same time as those growing under natural conditions. The archegonia begin to develop in April in the same thalli which have

produced antheridia, and all stages are found by May 1. About this time fertilization takes place, and by May 25 all stages of sporophyte are found. The arrangement of the sexual organs in the thallus is shown by *figs. 7–11*.

The vegetative growth is very rapid during April and May, the thallus becoming broad and branched by the increase in the number of growing points. The narrow older part by which the thallus is attached decays, and the younger part bearing the sexual organs and sporophytes is set free and floats upon the water. When the plants are supplied with a large amount of water changes take place in the lamellae. They grow to great size and become purple. In the floating thallus decay of the older part continues; the part bearing the antheridia first disappears, then the part bearing the sporophytes, and finally the growing points may be separated, one thallus thus giving rise to several new individuals. In most cases observed the decay of the older parts in floating plants did not advance so far. The plants were carried up around the edge of the pond by the waves, and as the water went down were left stranded upon the mud. When the thalli settle down upon the mud, the large ventral plates wither, and rhizoids are put forth which in a few days attach the thallus to the soil. Growth now continues at the growing points, so that new branches are produced which form rosettes.

When the thallus is injured at this time new plants are immediately produced from the cells of the apical region. This was first observed in plants injured by being covered with mud, in which case slender delicate outgrowths were produced (*fig. 4*). Other plants injured by snails soon developed long slender plants (*fig. 3b*). Thalli were cut into pieces to determine whether other cells would show the same plasticity, but new plants were produced only from cells near the growing point. VÖCHTING (**33**) found in Lunularia that regeneration takes place from cells in various parts of the thallus, but this does not seem to be true of *Riccia natans* under the conditions in which I have studied it. Large numbers of the plants which were left upon the mud when the water went down were injured by cattle coming down to the ponds to drink. Later in the season the cattle tracks were lined with young, green, ribbon-shaped plants

which were outgrowths from the growing points of the older plants. The cattle tracks serve a good purpose, as the young delicate plants are shaded and protected to some extent during the dry season. Two forms of the thallus are produced by the different methods of propagation. In the one case the thallus after it becomes attached to the soil continues its growth, branches and forms a rosette, while in the other case the thallus is injured, and very delicate forms are produced. When large numbers of the floating thalli are deposited near together and are then injured. we find the irregular clusters of plants which have been described in the first paragraph.

The thallus of this plant during the floating period bears such a striking resemblance to *Ricciocarpus natans* that one is led to the conclusion that *Riccia lutescens* is only a ground form of *Ricciocarpus natans*. Since the beginning of this study and after it was well under way, a paper was published by GARBER (**11**) which dealt with the life history of *Ricciocarpus natans*. Several points in the biology of the plant as given by GARBER differ from those found to obtain at Ithaca, and since the structure of the thallus as well as the embryology is conclusive proof that the two forms are the same species, it seems proper to call attention to these differences and then to give briefly the embryology before taking up the other phases of the study.

The greatest difference in our observations lies in the relation of the supply of water to sexual reproduction. GARBER states that *Ricciocarpus natans* as it grows at Chicago spends its entire life, from the germination of the spore to the production of spores, in the floating state, and that the occasional fruiting plants found upon the soil in summer are plants in which the sexual organs developed and the sporophytes began their development while the plants were floating. He observed no case in which sexual organs were produced on plants growing upon the soil and states that *Ricciocarpus natans* has not yet acquired the power to reproduce sexually when growing upon the soil. The sexual organs develop in April.

The plants at Ithaca, however, spend the greater part of their life upon the soil and only float upon the water for a few weeks at the fruiting period. The sexual organs begin to develop in autumn while the plants are on the soil and plants kept on the soil and sup-

plied with a limited amount of water developed fruit. At the time when the antheridia begin to develop the gametophyte is under favorable conditions for vegetative growth, but is not supplied with an abundance of water. The soil is moist and the conditions are such as would favor the growth of a terrestrial form like Marchantia.

The plants seem especially adapted to spend the winter submerged and do not perish under such conditions. The fore part of the thallus contains very large air cavities and thus the tissue is aerated. It is well known that certain higher plants which grow in wet situations have large air spaces in the tissue, and GANONG (12) calls attention to the fact that those marsh plants which are submerged for a portion of the year are able to survive on account of their capacity for air storage. About May 1 the older part of the thallus, which is narrow and thin, has decayed, and the younger parts, bearing the sexual organs, is set free and floats. GARBER points out that when land forms are placed upon the water only a small portion of the apical end remains above the surface, while the older part of the thallus extends into the water and decays. This is true of plants taken from the soil in summer, but in the spring when the free part of the thallus is thick and contains large air cavities, it floats readily. The length of the floating period depends of course upon the conditions of the pond. In some cases the plants may very soon be carried up around the edge of the pond and deposited on the mud, but floating forms are usually found until the ponds are almost dry. In the case of ponds which do not become dry in summer, both forms would be found. The floating period affords an excellent means for distribution.

When the plants grow upon the soil and are not protected during the winter by a covering of snow or water, they are usually killed by freezing, but in some cases plants which were brown and seemed to be dead produced new thalli from the growing point. The young delicate thalli are well adapted to tide over the dry season, because they can live with a less supply of water than would be needed by the older plants.

RELATIONSHIP OF THE SPECIES.

The form usually described as *Riccia lutescens* should be regarded as a ground form of *Ricciocarpus natans*. Both in the field and in

cultures in the laboratory the forms have been observed showing the transition. There can be no doubt that the plant which I have described is the true *Ricciocarpus natans*, and the description of the ground form as a distinct species came about naturally from the conditions of its growth. In such ponds as have been observed here, the water is high in April and May, so that the floating plants are carried up around the edge and left on the soil. In June or July the water has entirely disappeared from the pond and the only plants found are the slender ribbon-shaped ones which have developed from the floating form. In my first summer's collecting, when the ponds were dry by the last of June, I saw not a vestige of the old *Ricciocarpus natans*, and felt sure that the plants collected were *Riccia lutescens*. It seems possible that the plant was first described as a distinct species from material collected under similar conditions, because it is said to occur in dried up ponds and ditches. If in summer and autumn some water were present, so that some of the typical *Ricciocarpus natans* would be found floating, the origin of the ground form might readily be seen, but in such a case there might be failure to associate the ground form with *Riccia lutescens*. Only by following the development and observing the transition of one form into the other under different conditions of growth can the true relationship be determined. My observations have convinced me that *Riccia lutescens* is only a ground form of *Ricciocarpus natans* and should not be regarded as a distinct species.[2]

The plant now known as *Ricciocarpus natans* was formerly regarded as a Riccia. In the structure of the thallus Ricciocarpus is more complicated than the species of Riccia. The most important taxonomic characters, however, have been the arrangement of the sexual organs and structure of the sporophyte.

HOOKER first found fruiting plants in dry specimens sent to him by TORREY from New York in 1824. BISCHOFF found fruiting plants in the autumn of 1829 near Heidelberg, and describes antheridial plants, but his figure of the antheridium is not very convincing,

[2] Having determined the ground form as *Riccia lutescens*, specimens were sent to Professor A. W. EVANS in October 1904. He considered that we were right in referring the plants to that species, but stated the views of different authorities in regard to the status of the species.

as it looks more like the mass of tissue which projects up as a ridge into the median groove, the cells being quite too large for those of an antheridium.

Although HOOKER considered that the plant should remain in the genus Riccia, CORDA placed it in a new genus, Ricciocarpus, on the basis of HOOKER'S description and figures which were taken from dried material. CORDA'S figures are copies of HOOKER'S. BISCHOFF held that there was no real basis for the change, as the mature sporophyte does not differ from that of other Riccias, the separation being based on the mistaken notion that the capsule walls disappear entirely at maturity, and that the genus Riccia should not be divided on account of differences in the thallus brought about by the different conditions under which the plant grows, since the method of fruiting is the same in all the species.

LEITGEB regarded Ricciocarpus as a distinct genus, on account of the more complex structure of the thallus and the grouping of the sexual organs. He thought that the antheridia were collected into groups similar to those in the Marchantiaceae, but GARBER'S results and my own show that LEITGEB was not correct, and that the antheridia actually form only one group. The archegonia are also arranged in a definite part of the plant in one group.

The question now arises whether this is a more advanced condition of development than is found in species of Riccia. In the lower species of Riccia, the sexual organs are said to be indiscriminately scattered over the surface of the thallus, while in *Riccia fluitans* a regular alternation of single antheridia and archegonia is described. CAMPBELL, in discussing the arrangement of sex organs in Riccia, says that in the two forms which he studied, *Riccia hirta* and *Riccia glauca*, he found as a rule that several of one sort or the other would be formed in succession. I have observed the same in *Riccia crystallina*, although the older sporophytes appear scattered in the thallus. LINDENBERG described the fruit of *Riccia crystallina* as scattered, but the antheridia are described and figured as being in a group along the middle part of the thallus. He described and figured the fruit in *Riccia glauca* as being sometimes in rows and sometimes scattered. Most of the figures show them in more or less perfect rows along the longitudinal axis.

In *Riccia minima*, LINDENBERG (**20,** p. 429) describes and in *pl. 20* figures the antheridia as arranged in two rows, one on each side of a median groove. In *Riccia bulbosa* the antheridia are along the median groove for its entire length, sometimes in pairs and sometimes far apart. *Riccia Bischoffii* has the antheridia in two or three rows in the thallus.

It seems highly probable that a careful study of a large number of species of Riccia by modern methods would show that in many of them there are produced groups of antheridia and archegonia in distinct parts of the thallus.

Since the characters upon which the genus Ricciocarpus has been based, with the single exception of the structure of the thallus, have been found wanting, it seems to me that there is not sufficient reason for retaining the genus. The thallus varies in form according to the supply of water, and when growing on the soil has been called a species of Riccia. Many plants assume quite different forms when growing under different conditions, but the different forms are not regarded as species.

We should then write:

RICCIA NATANS L. Syst. Veget. 956. 1774.—Bischoff, Nova Acta Acad. Caes. Leop. Carol. **17**: 2. 1835.—Lindenberg, Nova Acta Acad. Caes. Leop. Carol. **18**:—1836.—Sullivant, Gray's Manual 2ed. 1856.

Ricciocarpus natans Corda, Opiz Naturalischentausch. 1829.—Leitgeb, Die Riccien, Unters. Lebermoose 4: 1879.—Lindberg, Revue Bryol. **9**:82. 1882. (Includes *Riccia natans* L. and *Riccia lutescens* Schw.)—Schiffner, Engler and Prantl. 1893.—Campbell, Mosses and Ferns. 1895.—Underwood, Systematic Botany of North America. Hepaticae. 1895.—Garber, BOT. GAZETTE **37**:101–177. *pls. 9–10.* 1904.

Riccia lutescens Schw. Specimen Fl. Amer. Sept. Crypt. 26. 1821.—Lindenberg, Nova Acta Acad. Caes. Leop. Carol. **18**: *pl. 26.* 1836.—Sullivant, Mem. Amer. Acad. II. **4**: *pl. 4.* 1849.—Sullivant, 2d ed. Gray's Manual 684. 1856.—Underwood, Systematic Botany of North America, Hepaticae. 1895.

Riccia velutina Hooker (in part) Ic. Pl. *pl. 149*: founded on sterlile thalli of *Riccia lutescens* and fertile thalli of *Riccia crystallina*, according to Sullivant, Gray's Manual, 1856.

EMBRYOLOGY.

Material for study was collected during the autumn and spring, and fixed very satisfactorily in 1 per cent. chromacetic acid or in chromosmacetic.

The large air cavities prevent the penetration of the fixing fluid, to overcome which the pieces were submerged by means of cotton plugs. After dehydration the material was passed through chloroform into paraffin. Sections were stained with the triple stain of Flemming or with Heidenhain's iron-alum haematoxylin.

SEXUAL ORGANS.

Young antheridia were found in October. They begin to develop while the plants are young and growing on soil not supplied with a large quantity of water, although the conditions for vegetative growth are good. At this time the thallus is ribbon-shaped, with a thickened apical end and a longitudinal median groove, the thallus in cross-section having about the shape of an inverted Y with a ridge of tissue between the arms (*fig. 9*). Very few plants are found which do not produce antheridia. The archegonia develop later in the same thallus. At first there seemed to be in this a distinction between *Riccia lutescens* and *Ricciocarpus natans*, because *Ricciocarpus natans* has been described by SCHIFFNER, LEITGEB, and CAMPBELL as being strictly dioecious, but the work of GARBER shows conclusively that it is monoecious. The earlier observers state that Ricciocarpus fruits in autumn, so it seems probable that their material was collected after the older portion of the plant had decayed, leaving only the portion bearing sporophytes.

The antheridia are produced in acropetal succession in three to five rows (*figs. 10, 11*).

The antheridium develops as has been described for other species of Riccia. A superficial cell on the floor of the dorsal furrow just back of an apical cell protrudes above the surface and is cut off by a horizontal wall. The outer cell increases in size, and is divided by three or four cross walls, then a longitudinal wall is formed dividing the young antheridium into two equal parts: this is followed by a second longitudinal wall perpendicular to the first. Then periclinal walls are formed which cut off the single layer of cells which form the wall of the antheridium. The cells in the center now undergo repeated divisions until a very large number of cells is formed. Each of these cells is almost cubical in form and in Riccia has been described as producing a single spermatozoid,

KNY (**19**). The mature antheridium is a short stalked oval body with a conical apex.

As the antheridium develops, the vegetative tissue grows up and surrounds it so that it is enclosed in a cavity which opens into the dorsal furrow. This cavity is formed in the same way as the air spaces of the thallus. The apex of the antheridium is a little below the floor of the dorsal furrow and the sperms escape through the neck formed by the surrounding tissue. Although the antheridia begin to develop in autumn, they are not mature until the following spring, because the growth is checked by the cold. Plants kept in a warm place produced mature antheridia during the winter.

A series of archegonia is developed which is a continuation of the series of antheridia (*fig.* 7). The archegonium is at first superficial on the floor of the dorsal furrow. Later it becomes enclosed in a cavity by the upward growth of the vegetative tissue as in the case of the antheridium except that the neck of the mature archegonium protrudes above the bottom of the furrow. The origin of the archegonia side by side at the bottom of the dorsal groove is shown in figure 9. In this way three to five rows are formed and later a large number of sporophytes are found in each thallus.

The archegonium develops in general as has been described by JANCZEWSKI (**17**). My observations confirm the account given by GARBER for *Ricciocarpus natans*, as a comparison of the figures will show, so it is unnecessary to describe the development here.

About the time when the archegonia are mature, cross-sections of the thallus show numerous, delicate, almost hyaline, club-shaped hairs extending up from the floor of the median groove. Each hair consists of a stalk of two or three short, narrow cells with a much larger cell at the free end. These hairs bear a striking resemblance to paraphyses(*fig.* 78). LEITGEB (**22,** p.31) describes "papillae" which grow up from the bottom of the groove and regards it as highly probable that it was the dried remnants of these which LINDENBERG observed when he wrote: "Sporangium vor aussen mit kleinen unregelmässigen braunen Schuppen bedeckt ist, die Fragmente einer zersprengten früheren Hülle zu sein scheinen." As the hairs become older they become brown and break down so that they

would give much the appearance described by LINDENBERG. We know now that the sporophyte has no *Hülle* or sheath.

SPOROPHYTE.

The development of the sporophyte agrees with the account given by CAMPBELL (**3**) for Riccia and by GARBER for *Ricciocarpus natans*.

The first division is usually transverse but may be oblique (*fig. 21*). The next wall may be perpendicular to the first so as to form a quadrant (*fig. 22*), or parallel to it, producing a row of cells. Divisions take place in all directions after this until an almost spherical mass containing several cells is formed. Then the amphithecium becomes distinct as a single layer of cells enclosing the spore producing cells. The growth takes place rapidly but the divisions of the cells are not simultaneous, usually only a few dividing cells being found in a sporophyte.

The sporophyte continues its growth until a solid mass of three to four hundred cells is produced. Then the calyptra and amphithecium expand and the spore mother-cells becoming free separate from one another and become rounded. From the surrounding cells, which are richly stored with food, there is secreted a large amount of nutritive material which fills the space around the mother-cells, giving them favorable conditions for growth (*figs. 25, 26*). The spore mother-cells increase rapidly in size and again fill the cavity. That part of the nutritive material not absorbed by the spore mother-cells is pressed into thin plates between them. This material takes a deep blue stain with gentian violet. A fuller discussion of the spore mother-cells and of their division to produce the spores will be given in another place.

Before the spores are mature the inner layer of the calyptra collapses. The amphithecium is distinguishable until the spores are almost mature. The outer layer persists but the cells are usually shrunken. The contents of these cells is no doubt used up to supply the growing spores with nourishment. All of the spore mother-cells produce spores, there being no sterile tissue in the form of elaters. In discussing the simple form of sporophyte of Riccia, GARBER considers that the absence of sterile tissue is to be associated with the habit of the plants; since there is not much chance for the attachment of an independent sporophyte, there is no sterile tissue in

the form of a foot. When we consider the fact that some other Hepaticae which have the foot well developed grow on very wet soil and require as much moisture for their development as do some of the species of Riccia, this theory does not seem entirely convincing.

The sporophyte develops during May and June. A given sporophyte requires about three weeks for its development.

SPOROGENESIS.

Usually the most favorable cells for the study of cytological details are the spore mother-cells. Their large size, abundant contents and active growth at the time when divisions are taking place, permit good results in fixation. *Riccia crystallina* has furnished the most satisfactory material.

In July, 1903, an abundance of fruiting *Riccia crystallina* was found growing on the mud on the bottom of a dried up pond not far from the ponds where the form known as *Riccia lutescens* was growing. This species had never been collected in this region before. Having so determined the plant, I referred specimens to Prof. A. W. EVANS who confirmed my determination. He says: "Apparently this species represents an addition to the hepatic flora of New York. I find no mention of it in local lists of New York plants and there are no specimens of it from your state in my herbarium."

These plants had been growing under favorable conditions, as the pond had not contained much water at any time during the spring. The thalli formed rosettes growing so close together as almost to cover the ground. The number of fruiting plants was very striking, as it seemed impossible to find a single sterile plant. All stages in the development of the sporogonium and spores were easily obtained, and some stages in the development of the sexual organs, but changes were taking place very rapidly and the younger stages were of comparatively rare occurrence. The development of the sexual organs and fruit agrees with that of other species of Riccia. Each thallus produces several sporophytes which are easily recognized when mature as small black spherical bodies imbedded in the tissue.

These plants continued to develop and produce sporophytes for only a short time after they were discovered. The month of July

was the most favorable time for the collection of material showing karyokinesis in the spore mother-cells. During August, the spores became mature and the thalli broke down. No good specimens could be collected after August 25. This differs from what has been observed for some other species of Riccia, which are described as withstanding long periods of drought, the thalli continuing their growth again when supplied with moisture. (CAMPBELL, **3**.)

During the following winter this pond became filled with water and did not become dry until late in the summer, so that only a few plants were found as compared with the large number of the preceding year. This made a difference in the time of fruiting. In September the sporophytes were in about the same stage of development as in July of the preceding year. This may explain why different authors give different seasons for the fruiting of Riccia. It seems that conditions of temperature and water supply exert such an influence that in the same species and locality the time may vary considerably from year to year. In general, I think it may be said that good conditions for vegetative growth will hasten rather than retard the fruiting of Riccia.

The thallus of *Riccia crystallina* is small and thin; its surface presents a series of wide depressions separated by thin lamellae; and there are no ventral scales. The fixing fluid easily penetrates and the spore mother-cells are usually well fixed.

The development of the spore mother-cells agrees with the account given for *Riccia natans*, but there is not such a large number produced in a sporogonium. When the spore mother-cells come to lie loosely in the sporogonium, they are surrounded by nutritive material. The mature spore mother-cells are then generally spherical, but they may be elliptical or so angular by crowding as to look like a tissue. The contents of the spore mother-cells of Riccia has been described as granular by CAMPBELL (**3**) but the structure of the cytoplasm in *Riccia crystallina* is a fine reticulum with the granules occurring usually at points of intersection of the fine threads of the network. The older spore mother-cells as well as the mature spores contain considerable oil.

In the nucleus of the spore mother-cell the chromatin is scanty and is irregularly scattered on a fine linin network. No nucleolus

has ever been observed (*fig. 34*). When the nucleus is preparing for division, the chromatin leaves the linin network and collects into several bodies which soon move together to form one irregular mass. I regard this as the synapsis stage. Such bodies of chromatin have been found often and in cells which seemed to be well fixed so it seems to represent a stage in the preparation for division and not to be a result of shrinkage as has been suggested by certain authors for other plant cells in which the same condition has been observed. The body of chromatin occupies a position at one side of the nucleus, and the rather large nuclear cavity appears hyaline. There can be little doubt that the body described by CAMPBELL (**3**) as a nucleolus is really the entire mass of chromatin in the synapsis stage.

From this mass of chromatin a short thread develops which later segments to produce the chromosomes (*fig. 35*). The small amount of chromatin present here makes the details very difficult to determine. The four chromosomes, easily counted here as well as in the nuclear plate and on the way to the poles, are very small and appear almost spherical when on the spindle although they are short, thick, curved rods.

The development of the spindle is not easily observed. Divisions take place almost simultaneously in all the cells of a sporogonium and the changes are very rapid. By far the commonest stage of division is that in which the chromosomes are in the nuclear plate (*fig. 38*). Neither centrosomes nor centrospheres occur in the spore mother-cells of either *Riccia crystallina* or *Riccia natans*. Around the nucleus preceding the formation of the spindle, there is an accumulation of material, apparently composed of fine fibres. The nucleus elongates, becoming somewhat elliptical but not sharp pointed. The fibres about the nucleus do not give the appearance of centrospheres but are like the weft of kinoplasmic fibres described for certain pollen mother-cells (*figs. 36, 37*). It has been impossible to find any nucleus which showed anything resembling a multipolar spindle. The poles of the spindle are probably determined by the elongation of the nucleus at an early stage in the spindle formation. The spindle is composed of very fine fibres, some of which extend from pole to pole, while others extend from the poles into the cytoplasm, reaching almost to the nuclear plate (*fig. 38*). The

mature spindle has very broad poles and its formation does not seem to have been controlled by a centrosome or a centrosphere, as a comparison of the spindles of the spore mother-cells with those of the cells of the antheridium makes clear.

The minute chromosomes separate, four going to each pole, after which a cell plate is formed in the usual way (*figs. 40, 41*). The daughter nuclei do not come to a true resting stage. The chromatin is scattered in almost spherical bodies in the hyaline cavity of the nucleus, which do not represent the individual chromosomes, as their number and size vary considerably (*fig. 42*).

The second division takes place in much the same manner as the first. The spindles are arranged with their long axes parallel to the first cell plate, so that the cell plates formed in these spindles are almost perpendicular to that formed in the first division (*figs. 43–47*). The latter does not disappear during the second division but remains and the walls separating the spores are laid down here (*fig. 47*). The walls separating the cells of the young tetrad are thin and delicate, but in the mature spore the outer layer of the wall becomes thickened and folded. The mature spore is almost black, and its contents are largely oil. When carried through chloroform into paraffin and sectioned, the spores seem to have only scanty granular contents, due to the fact that the oil has been removed in the process. The nucleus is very small.

During the winter and spring following their development, unsuccessful attempts were made to germinate the spores. It may be that they had been allowed to remain dry too long before they were moistened, for in nature they would not be dry very long even in tiding over a dry season.

The spore mother-cells of *Riccia natans* do not furnish such satisfactory material for study as do those of *Riccia crystallina*, because the large air cavities of the thallus prevent the penetration of the fixing fluid and so the spore mother-cells often shrunk. A sufficient number of good preparations was secured, however, to show that the process of division does not differ from that of *Riccia crystallina*.

SPERMATOGENESIS.

The development of the spermatozoids has been treated by a number of investigators, among them, CAMPBELL (**4**), LECLERC DU SABLON (**23**), GUIGNARD (**14**), SCHOTTLANDER (**27**), and STRASBURGER (**29**). It will be observed that most of these papers were published before methods of preparing material for study were so well developed as at present. The work of BELAJEFF (**1**) confirmed by that of STRASBURGER (**29**) shows that the spermatozoid in the Hepaticae consists not only of the metamorphosed nucleus but also of the cytoplasm.

IKENO (**16**) not only confirms the view that the spermatozoid consists of cytoplasm as well as nucleus but also discusses the development of the cilia and the homology of the blepharoplast and centrosome of *Marchantia polymorpha*.

He finds that the body which becomes a blepharoplast in the developing spermatozoid appears in the earlier nuclear divisions of the antheridium and functions as a centrosome. It is, however, not permanent, but appears at the time of nuclear division and disappears during the process, so that it is not found in the daughter cells until about the time for the formation of the spindles of their division. After the last division which gives rise to the cells that develop into the spermatozoids, the body does not disappear but remains and becomes a blepharoplast. IKENO argues from this that the centrosome and blepharoplast are homologous. He has good grounds for such an argument in the case of *Marchantia polymorpha*, because centrosomes have been reported also in the vegetative cells of that plant, by MOTTIER (**24**) and by VAN HOOK (**32**). In other plants which have the blepharoplast, centrosomes are not found, and the body appears in only one or two divisions before the formation of the cells which produce the spermatozoids.

MOTTIER (**26**) in discussing IKENO'S paper raises the question whether the bodies which IKENO has figured as centrosomes are in some cases more than ordinary granules such as appear in the cytoplasm of other cells in which centrosomes are known to be absent. IKENO has pointed out, however, that the cytoplasm of these cells is very finely granular, there being no other bodies in the cell which bear any resemblance to the ones figured as centrosomes. He also calls

attention to the fact that centrospheres have been described in dividing spore mother-cells of *Pellia epiphylla*, by FARMER (**6, 7, 8, 10**) and by DAVIS (**5**). The occurrence of centrospheres here has been questioned, however, by GRÉGOIRE (**13**). In a recent paper, FARMER (**9**) reports centrospheres and occasional centrosomes in the spore mother-cells of *Aneura pinguis*.

In order to get good results in *Riccia natans* it is necessary to fix the material when growing rapidly. About equally good results were secured with chromacetic acid and with Flemming's weaker solution. The sections were stained with anilin safranin and gentian violet. It was found best to stain deeply in gentian violet and then to wash out carefully. In this way all details can be brought out clearly, although IKENO did not find it good for Marchantia.

The development of the antheridium has been described. When almost mature it consists of a large central mass of cubical cells surrounded by a wall one cell in thickness (*fig. 33*). In preparations from plants in which some antheridia are mature, one finds several stages in the development. The nuclear divisions do not take place simultaneously throughout an antheridium but usually all the cells of one of the segments marked out by the first walls dividing the antheridium, show the nuclei in the same stage of karyokinesis. In the most favorable preparations, therefore, one may find several stages of division in the same antheridium.

The cells of the young antheridium are almost cubical, with finely granular cytoplasm. The nucleus is rarely exactly spherical and has a rather thick membrane. The chromatin is in an irregular central mass, made up of a number of pieces. A nucleolus cannot be distinguished. The cavity surrounding the chromatin is large and hyaline (*figs. 53, 54*). In some cases a large number of small bodies of chromatin were found scattered irregularly in the nuclear cavity. The number of chromosomes is four. It seems that the nuclei in the young rapidly growing antheridium rarely come to a typical resting stage.

The question of the presence or absence of centrosomes in the cells of the young antheridium was taken up carefully, because previous observations on the karyokinetic figures in the sporophyte cells and spore mother-cells have convinced me that no such body

appears there. On the other hand centrosome-like bodies appear in the cells of the older antheridia at the time of nuclear division. There can be no doubt that these are distinct bodies, and they cannot possibly be interpreted as accidental granules in that position. In some of my preparations hundreds of cells showing them are found on a single slide, and they are so distinct that the preparation could easily be used for class demonstration. These bodies appear in the cells of the antheridium in early stages of its development. I have been unable to determine whether they appear in the earliest cell divisions but they appear in the antheridia which consist of only a few cells. They are not permanent, but disappear and arise anew with each division.

IKENO regarded it as highly probable (though unable to state this positively) that in Marchantia these bodies were of nuclear origin. He figures a small spherical body inside the nuclear membrane, which in a later stage is found outside the membrane. This body then divides into two, which arrange themselves on opposite sides of the nucleus. If the bodies have their origin as one, which later divides as described, they act as do the centrosomes which have been described for other plants.

In *Riccia natans*, nothing has been observed to indicate that the body is of nuclear origin, except that it stains in much the same way as the mass of chromatin in the nucleus. In some of my preparations a single body has been observed near the nuclear membrane (*fig. 53*). These bodies have never seemed so distinct as the ones which appear at the opposite ends of the nucleus and in the poles of the spindle. There is a dark central part, surrounded by a mass of cytoplasm which is more or less irregular but does not give the appearance of distinct radiations such as are described in the centrospheres of certain plants.

When these single bodies were discovered, a careful search was made of the same preparations and of others in which the two bodies were on the opposite sides of the nucleus, in order to discover if possible the intermediate stages which it would seem should appear in such preparations. In cases in which two bodies have been observed, they have always been on opposite sides of the nucleus, or so nearly opposite that the spindle developing between them

might take the curved form shown in *fig. 60*. The origin of the two bodies is of importance in determining the homology of the centrosome and blepharoplast and will be discussed later.

Starting with the stage in which the centrosome-like bodies are on opposite sides of the nucleus, the nuclear division takes place in the following manner. At first the bodies are at a little distance from the nuclear membrane, then the nucleus elongates so that the membrane closely approaches the bodies, becoming somewhat pointed. At the same time one observes that there is a collection of kinoplasm at the poles of the nucleus and extending along the nuclear membrane for some distance. At this time the bodies at the poles do not show radiations in any direction, but are very distinct (*fig. 54*). The spindle is formed from the kinoplasm which has been described, and when formed consists of a few thick fibres which converge at the poles, so that the centrosome-like bodies occupy the position of true centrosomes. About the time when the spindle develops, the chromosomes are formed from the central mass of the nucleus and become arranged in the nuclear plate. They are closely crowded together in this stage, and not so easily counted as when they have moved to the poles. The photograph (*figs. 75, 76*) shows the dense mass formed by the chromosomes when arranged in the nuclear plate. It was impossible to determine how the division takes place in the chromosomes as they move to the poles. The changes take place so rapidly that stages are rarely found in which the chromosomes are on their way to the poles.

The centrosome-like bodies disappear during the division, but it is difficult to say at just what point. *Fig. 56*, a cell taken from an antheridium in which only one or two more divisions will take place, shows the centrosome-like bodies quite distinctly when the chromosomes are almost at the poles, but by the time the chromosomes are at the poles and before the daughter nuclei are formed, the bodies disappear (*fig. 57*).

These bodies are best seen in preparations which have been over-stained and washed out. In some cases my preparations were stained deeply enough to show the spindle and chromosomes well, but only an occasional spindle showed the bodies at the poles. When these slides were over-stained and carefully washed out, the bodies were brought out very distinctly in all cases.

After a large number of divisions has taken place the antheridium consists of nearly cubical cells, each of which has been considered by earlier investigators to produce a single spermatozoid. STRASBURGER (**30,** p. 482) says of *Marchantia polymorpha*: "Die Spezialmutterzellen der Spermatozoiden sind durch fortgesetzte, sich rechtwinklig schneidende Teilungsschnitte angelegt worden." CAMPBELL (**4**) describes and figures the spermatozoid mother-cell of Pellia as producing two spermatozoids. IKENO (**16**) discovered that in Marchantia each of the cubical cells undergoes another division in which the spindles are arranged diagonally, in the earlier divisions the long axis of the spindle being parallel to the long axis of the cell. In this last diagonal division no cell wall is formed between the daughter cells, each of which develops into a spermatozoid. Thus each of the cubical cells produces two spermatozoids instead of one. IKENO cites several cases in which two spermatozoids are produced from a single mother-cell and thinks that this is probably general in the liverworts and mosses.

JOHNSON (**18**) has described a diagonal division of the cubical cells of Monoclea, but he figures a wall separating the two parts of the cell and regards each three-cornered cell as the mother-cell of a spermatozoid. He does not give the details of nuclear division in the earlier stages of the antheridium nor in the formation of the spermatozoid mother-cells.

In the last division of the cells in the antheridium of *Riccia natans* the spindles are arranged diagonally as in Marchantia. This arrangement of the spindles is quite striking. They are larger than in the earlier divisions and the bodies at the poles are very distinct. In some cases the spindles are curved (*figs. 58–60*).

No wall is formed between the daughter cells, each of which develops into a spermatozoid. The centrosome-like bodies do not disappear after this division (*fig. 61*). They remain in the cells, at first near the nuclei. The daughter cells are contracted, occupying the central part of the cell cavity (*figs. 62, 63*). Soon the centrosome-like body moves away from the nucleus toward the end of the cell. Those in the two spermatids may be at the same end or at opposite ends (*figs. 63–67*). When the spermatid has become somewhat rounded, the centrosome-like body has taken its position in contact

with the cell membrane (*fig. 68*). When the cilia appear they are inserted in this very small body so that it comes to function as a blepharoplast. Its small size as compared with that of the cilia of the mature sperm makes it seem probable that some of the material for the growth of the cilia must be drawn from another source than the blepharoplast itself, although it disappears to such an extent that in the mature sperm it cannot be recognized as the point of insertion of the cilia.

The developing spermatozoids of *Riccia natans* do not remain enclosed in the mother-cells until they are mature, but at about the stage represented by *figs. 70, 71* the walls break down and the young spermatozoids lie free in the cavity of the antheridium. Here they seem to undergo considerable growth. The material for this growth is probably derived from the surrounding cells as they become collapsed in old antheridia.

The nucleus of the developing spermatozoid takes a position at one side of the cell and becomes homogeneous. It seems probable that other material than the chromatin of the spermatid nucleus must enter into this part of the spermatozoid, because it is very evident that the body contains more material than would be obtained from the chromatin alone. Soon the nucleus elongates, following the outline of the cell and becoming crescent-shaped (*figs. 71–73*). In some cases, a distinct vacuole occurs in the cytoplasm although this is not always the case (*figs. 71, 72*). The mature spermatozoid becomes long and slender and consists of the nucleus, the material of which seems to have increased in amount, a small amount of cytoplasm, and the cilia which are derived from the blepharoplast and in all probability from a part of the cytoplasm surrounding it. IKENO describes a spherical body which appears in the spermatids of Marchantia before the cilia begin to develop and disappears about the time that changes take place in the nucleus. It has been impossible to find such a body in Riccia, although it would seem, judging from IKENO's figures, that it could easily be seen if present.

The question of the homology of the blepharoplast and centrosome is one which it seems to me has not yet been settled. In Marchantia, where centrosomes have been reported in the vegetative cells as well as in the antheridium, the evidence seems good that the

centrosome and the blepharoplast are homologous, and this is the conclusion of IKENO. In all other plants in which blepharoplasts are known to occur, centrosome-like bodies are not present in any cell divisions except those immediately preceding the formation of the sperms. That centrosomes occur in liverworts in cells outside the antheridium is open to question. The conflicting reports of those who have investigated *Pellia epiphylla* make it clear that no distinct body occurs there which can be regarded as a centrosome, although aggregations of kinoplasm, called centrospheres by most authors, do occur.

In *Riccia natans*, it seems very evident that centrosomes do not occur in the divisions of the spore mother-cells. The spindle poles are broad, and there is not even a suggestion of a centrosphere such as has been described for Pellia. In the cells of the sporophyte GARBER reports centrospheres but no centrosomes. I have never been able to observe them in my preparations. When the spindle is fully formed there are no fibres radiating into the surrounding cytoplasm (*figs. 48–52*).

Although the thallus of *Riccia natans* does not present favorable material for cytological study, a number of cells showing nuclear division in the gametophyte have been observed near the growing point. The greatest difficulty here is the presence of numerous deeply staining granules in the cell. In some cases granules resembling centrosomes appear at the poles of the spindle, but they do not differ in appearance from the other granules of the cell, and it seems probable that their occurrence here is accidental.

Summing up, we find that in *Riccia natans* centrosomes are not found in the cells of the gametophyte, sporophyte, or spore mother-cells, but that bodies occur in the dividing cells of the antheridium which seem to function as centrosomes. In Riccia and Marchantia, the blepharoplasts certainly have much more the appearance of centrosomes than in any other plants in which blepharoplasts have been described. The bodies have every appearance of centrosomes when at the poles of the elongated nucleus or at the poles of the spindle. Perhaps the strongest objection to regarding these bodies as centrosomes lies in the fact that in *Riccia natans* they occur only in the cells of the antheridium, while the blepharoplasts reported

in other plants appear only in the last two generations of cells concerned in the formation of spermatozoids.

Those who argue in favor of the homology of the centrosome and blepharoplast certainly find their best evidence so far in the liverworts, but it seems to me that this evidence is not conclusive when the bodies occur only in cells of the antheridium.

In those plants in which centrosomes are known to occur, a single body divides to produce two, which arrange themselves on opposite sides of the nucleus (MOTTIER, **25**). IKENO has reported a similar condition in Marchantia. In *Riccia natans*, however, the evidence seems to favor the view that the two bodies arise anew with each division, appearing on opposite sides of the nucleus at the same time. In this respect they behave more like blepharoplasts.

MOTTIER (**26**) in discussing this question has called attention to the fact that it is questionable whether we can speak of organs as homologous which, as such, are without genetic continuity. The question as to whether true centrosomes have genetic continuity has not yet been decided, but it is probable that they do not in all cases.

SUMMARY.

1. *Riccia lutescens* and *Ricciocarpus natans* are forms of the same plant, the former occurring on the ground in summer and autumn when the ponds are dry, and the latter as a floating form. Either form can be changed into the other by altering the supply of water. Therefore, *Riccia lutescens* should not be regarded as a distinct species.

2. The genus Ricciocarpus has been based largely on characters which do not exist. In my opinion, the only real basis for separating it from Riccia is the more complex structure of the thallus. BISCHOFF did not regard this as a good character for the separation of the genus.

3. The plant is monoecious, antheridia and archegonia being produced in definite groups in the same thallus. The sexual organs appear in autumn when the thalli are growing on the ground and complete their development the following April. Abundance of water is not essential to sexual reproduction, as the plants fruit when kept growing on the soil and supplied with a limited amount of

water; therefore the ground form is not sterile, as was the opinion of LINDBERG and GARBER.

4. Plants which have been growing attached to the soil and have been submerged by the filling up of the ponds do not necessarily perish, but are adapted to spend the winter under water and then to break loose by the decay of the older part of the thallus and float upon the water in the spring.

5. The plants are propagated vegetatively by the separation of branches of the thallus, by the decay of the older part, and also by the growth of new plants from cells in the apical region.

6. The sexual organs and fruit of the two species studied agree in their development with the accounts given for the other species of Riccia. There is no rudimentary integument surrounding the archegonium or sporophyte of *Riccia natans*. The sporogonium of *Riccia natans* is larger than that of *Riccia crystallina* and produces a larger number of spores. The only sterile tissue in either is the amphithecium, a single layer of tabular cells surrounding the mass of spore mother-cells.

7. Centrosomes are not present in cells outside the antheridium nor would I interpret any structure observed in the cells of the sporophyte or the spore mother-cells as a centrosphere.

8. Bodies which resemble centrosomes, and which are considered to be true centrosomes by certain authors, occur in the cells of the antheridium. These bodies do not have genetic continuity, but arise *de novo* with each division. They do not disappear after the last division of antheridial cells but remain in the spermatids and later become blepharoplasts.

9. In the earlier divisions of cells in the antheridium, the spindle is arranged parallel to the long axis of the cell, but in the last division, the spindle is placed diagonally in the cell. No wall is formed between the two cells produced by this division, each of which becomes a spermatozoid. Thus two sperms are produced from each cuboidal cell.

10. In the developing sperm, the blepharoplast takes a position on the membrane of the cell and the two cilia grow from it, the nucleus becomes almost homogeneous in structure and crescent-shaped, almost enclosing the cytoplasm. The mature sperm consists of the

nucleus, the cytoplasm, and cilia which have received material for their growth from the blepharoplast and probably also from the material surrounding it.

11. The amount of chromatin in the nucleus is small. There is no nucleolus present unless the masses of chromatin which are found in nuclei which are undergoing repeated division be interpreted as nucleoli.

12. The number of chromosomes is four for the gametophyte and eight for the sporophyte.

13. The cytoplasm of the spore mother-cells appears to be a fine reticulum, in which are numerous granules usually located at the point of intersection of the fibres of the reticulum.

14. The mature spore contains a large quantity of oil together with a small amount of granular matter. The nucleus of the spore is very small.

In conclusion I wish to thank Professor GEO. F. ATKINSON and Dr. E. J. DURAND for valuable advice and assistance during the progress of this study.

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EXPLANATION OF PLATES V–IX.

All drawings, except *fig. 7*, were made with camera lucida. *Figs. 8–52*, with Bausch & Lomb oculars and objectives, as follows: *Figs. 8, 10, 11*, 1 in. ocular, $\frac{2}{3}$ objective; *9* and *25* 2 in. oc., $\frac{1}{6}$ obj.; *19, 21–24, 26, 32, 33*, 2 in. oc., $\frac{1}{12}$ obj.; *12–18, 20, 27–31, 34–52*, 1 in. oc., $\frac{1}{12}$ obj.; *Figs. 53–73* with Zeiss oc. 18, 2^{mm} apochromatic objective, 1.40 aperture.

The figures of plate VI were reduced slightly more than one-half in reproduction.

All figures are of *Riccia natans* except the spore mother-cells (*figs. 34–47*) which are of *Riccia crystallina*.

PLATE V.

FIG. 1. Rosette of plants growing on the soil; *a*, natural size; *b*, enlarged.

FIG. 2. Land plants growing in regular clusters.

FIG. 3. Two plants growing on soil, one of which has been injured and has grown out in an irregular way from the growing point.

FIG. 4. New plants growing from apical cells of old thalli.

FIG. 5. *a*, Decay of older part of thallus of the land form to give the floating form; *b*, plants collected in May. If these thalli should become stranded on the mud and growth should continue rosettes would be formed.

FIG. 6. Plants decolorized in alcohol. The sporophytes appear as chains of dark bodies in the thallus.

PLATE VI.

FIG. 7. Longitudinal section of thallus parallel to the dorsal furrow, showing arrangement of sexual organs.

FIG. 8. Cross-section of thallus showing archegonia.

FIG. 9. Cross-section of thallus, showing the origin of archegonia in rows on floor of dorsal groove.

FIG. 10. Cross-section of thallus, antheridia.

FIG. 11. Longitudinal section of thallus parallel to surface, showing the arrangement of antheridia. Archegonia have not begun to develop.

FIGS. 12–18. Stages in development of archegonium.

FIG. 19. Archegonium in which egg-cell has not been fertilized and is shrunken.

FIG. 20. Cross-section of neck of archegonium.

FIG. 21–25. Stages in development of sporophyte.

FIG. 26. Spore mother-cells.

FIGS. 27–33. Development of antheridium. *Figs.* 27–31, from material collected in October.

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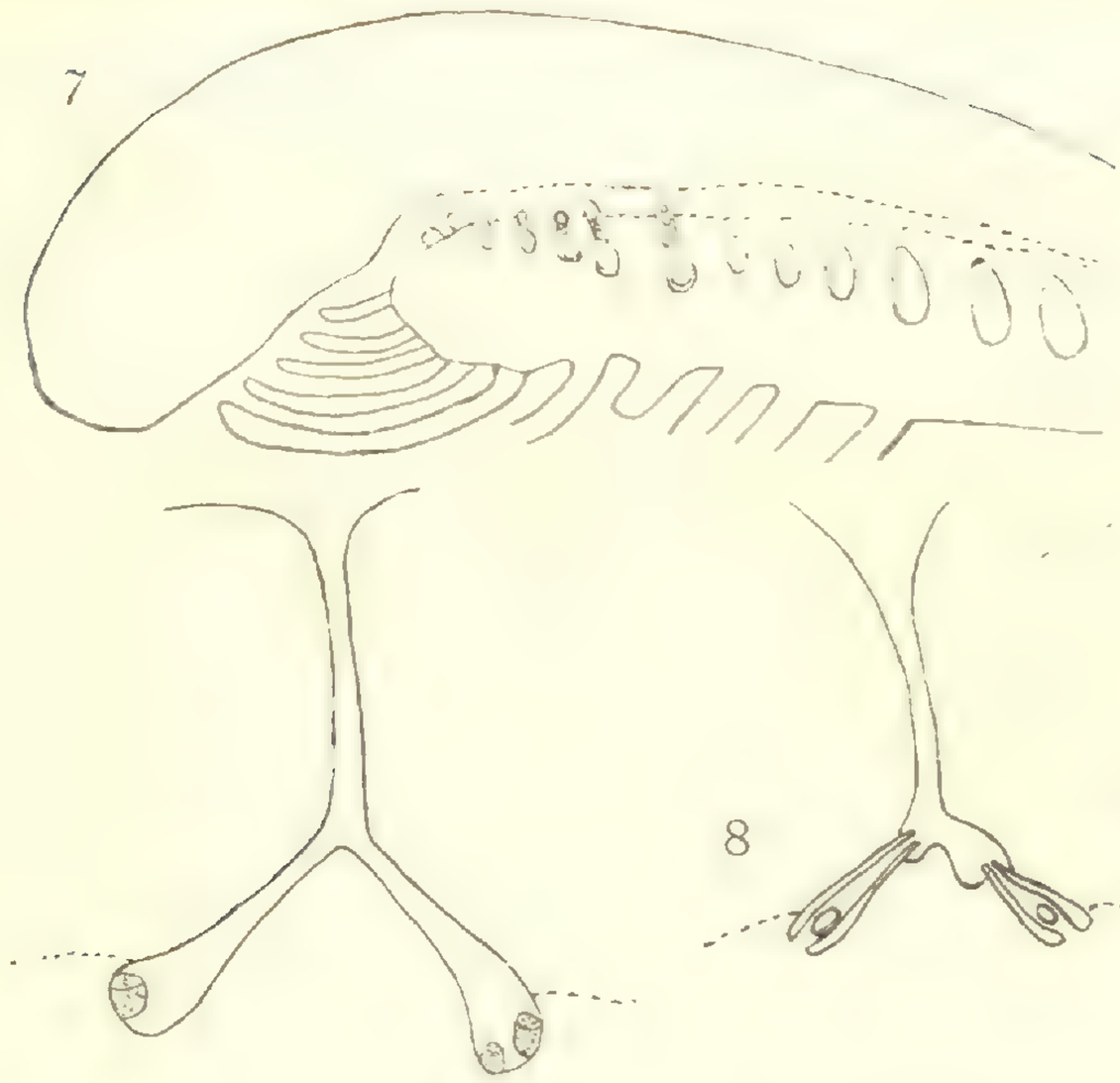
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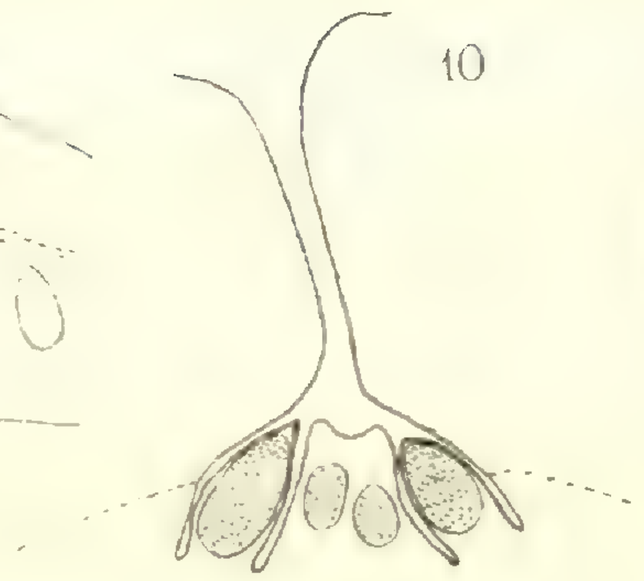
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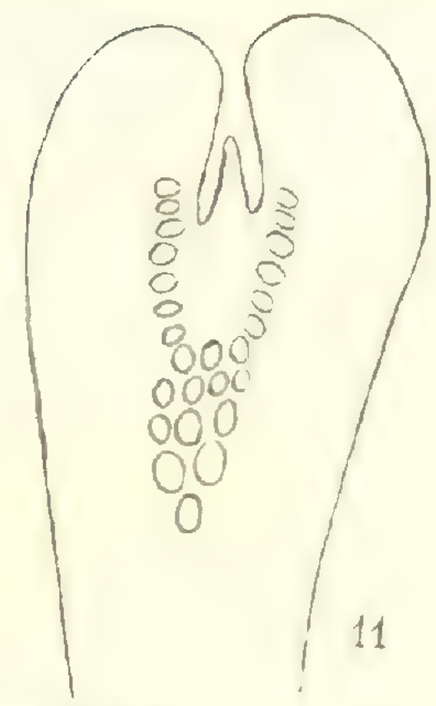
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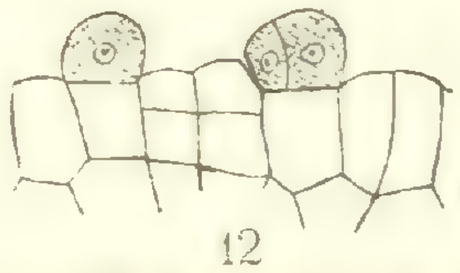
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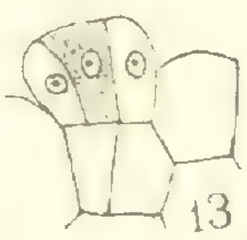
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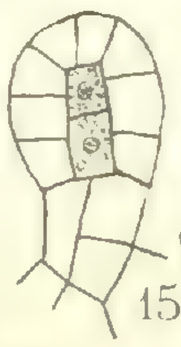
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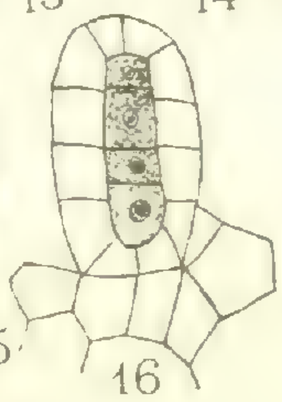
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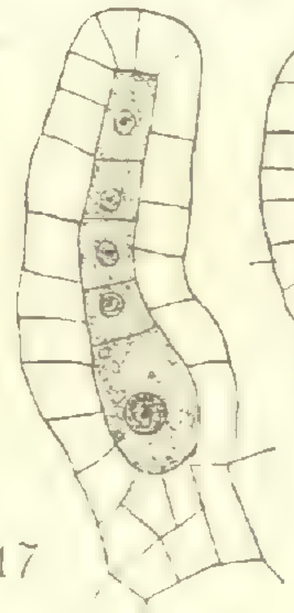
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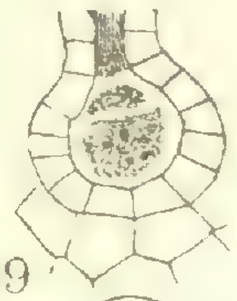
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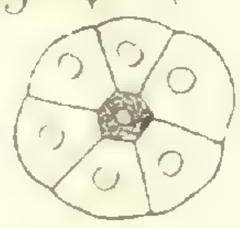
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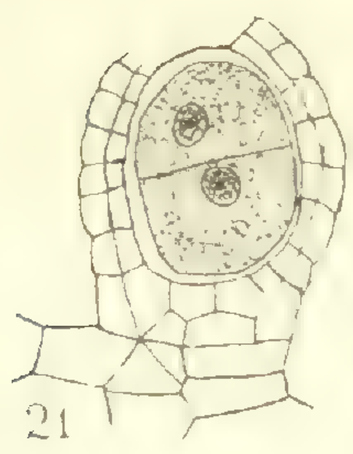
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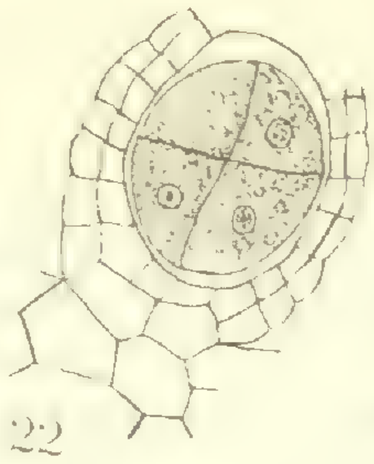
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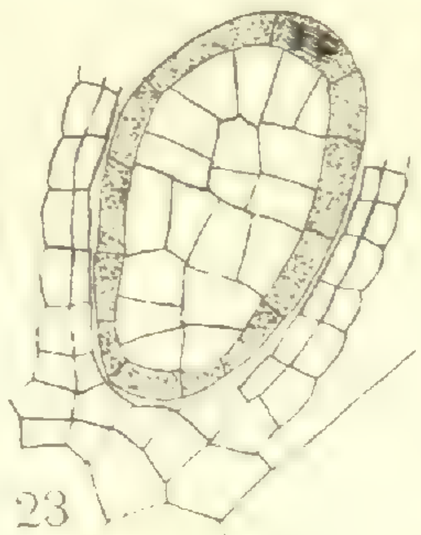
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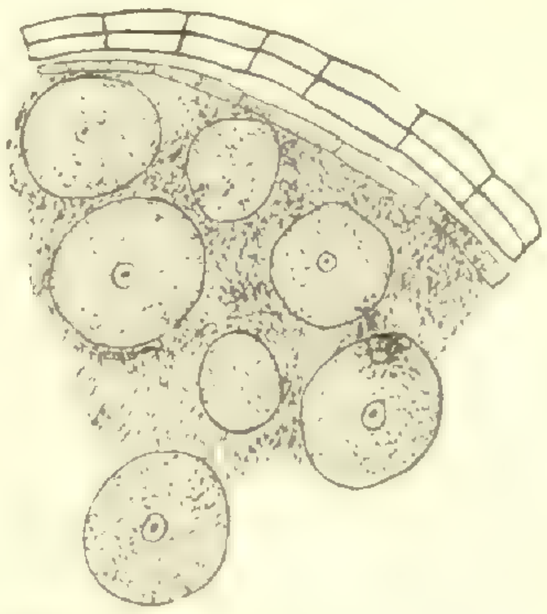
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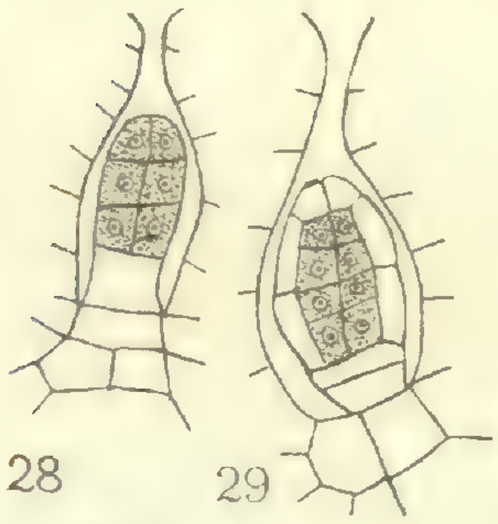
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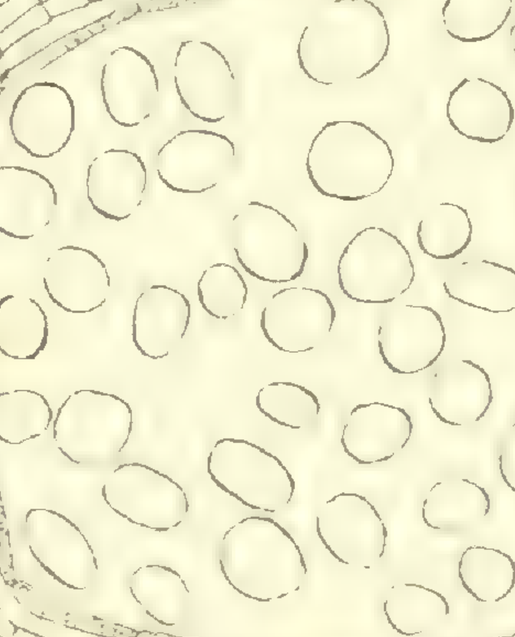
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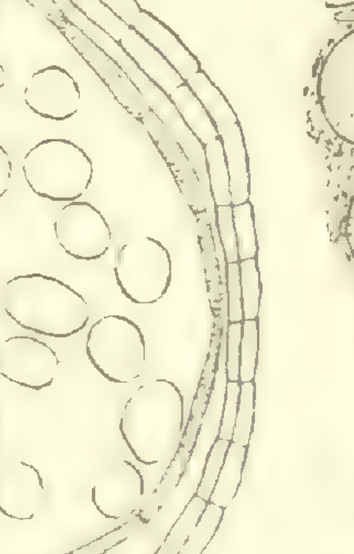
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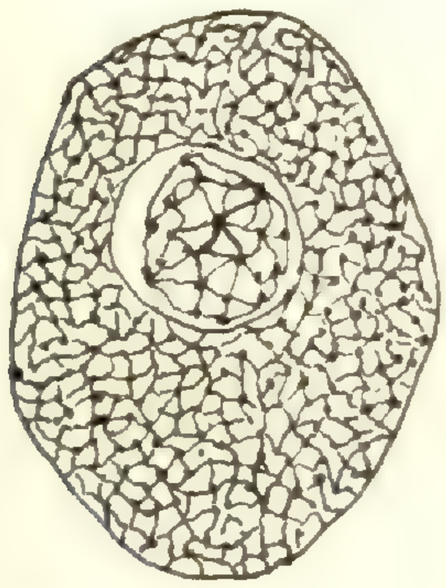

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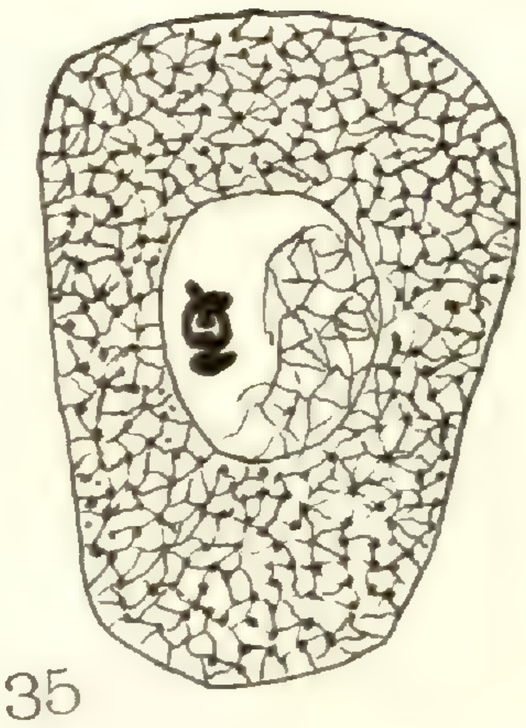

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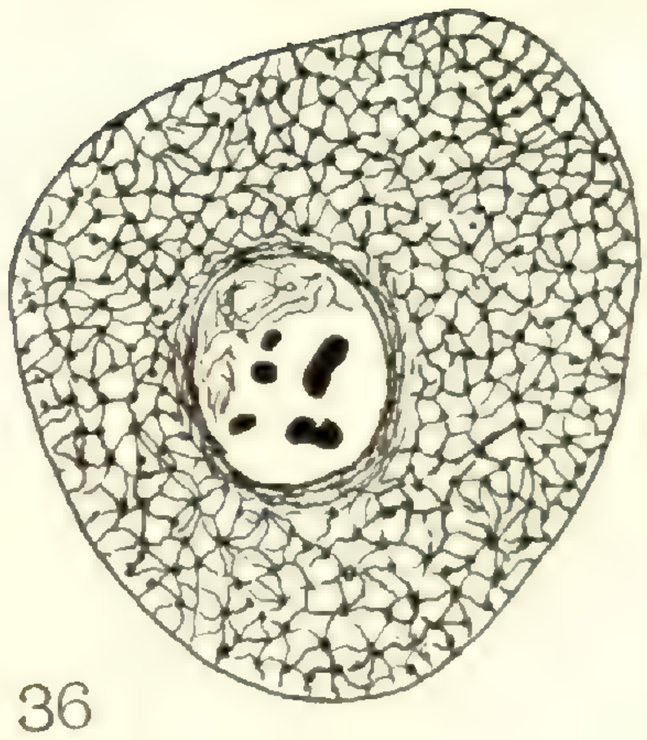

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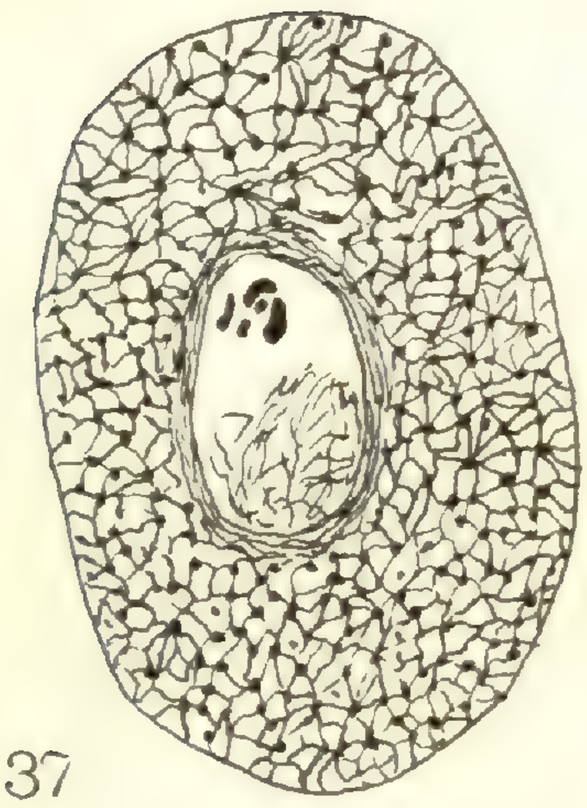

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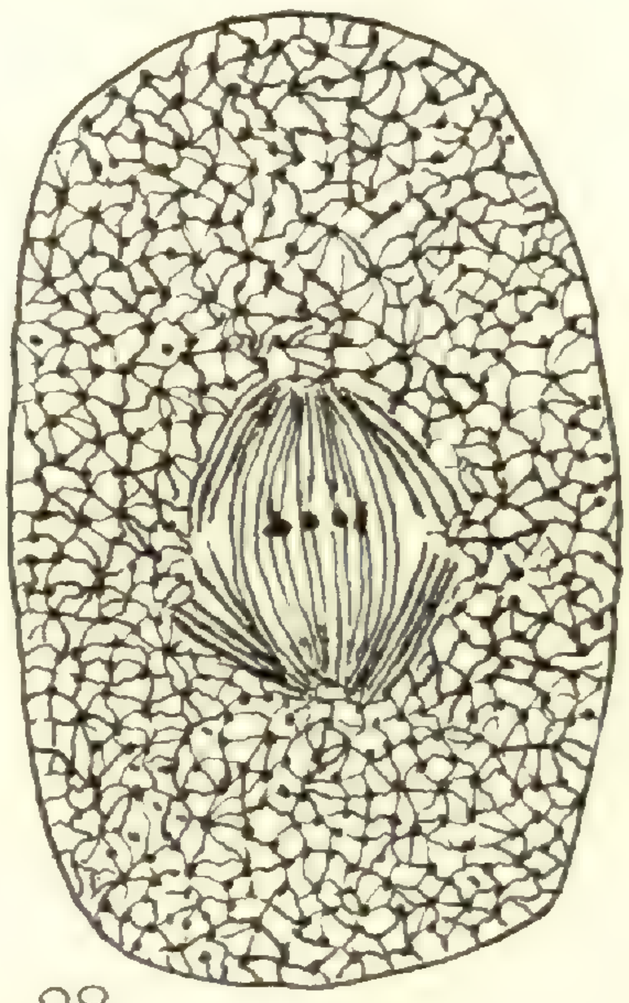

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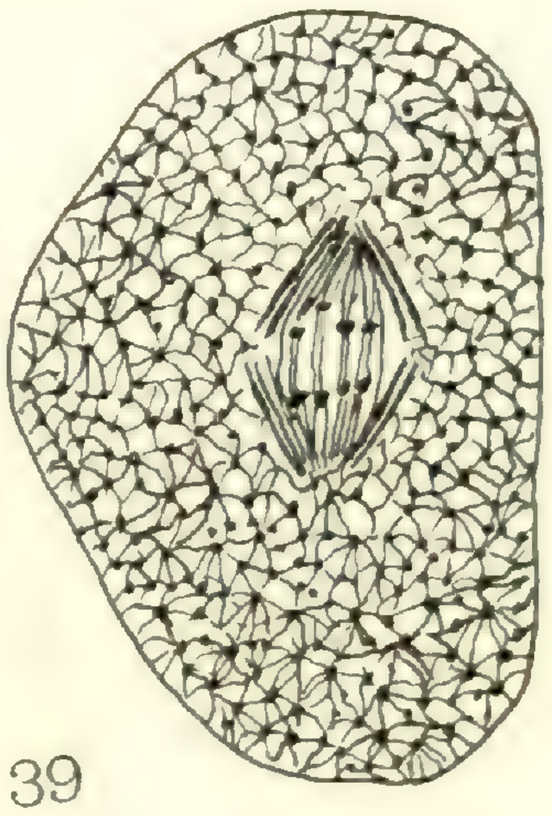

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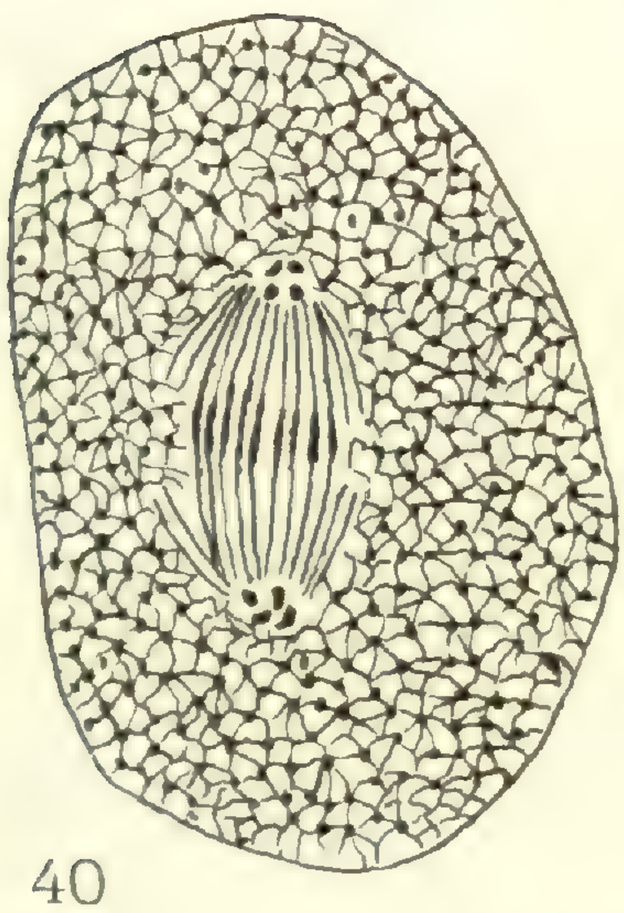

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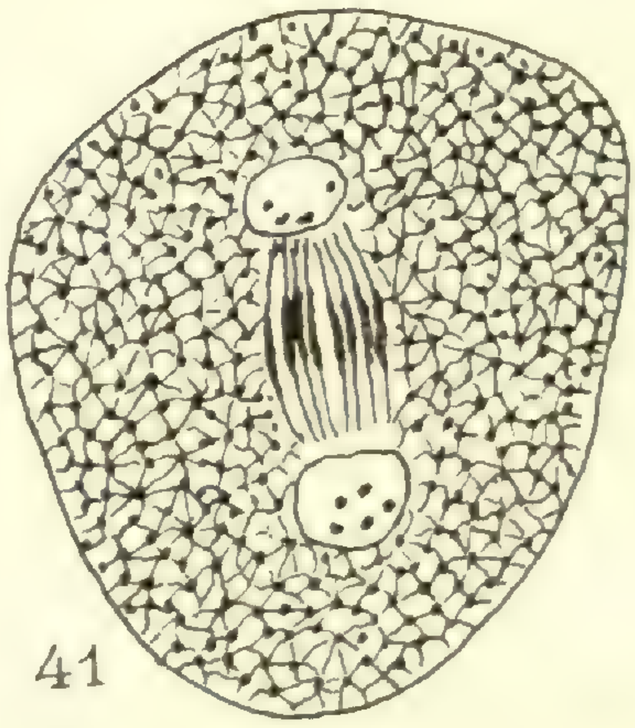

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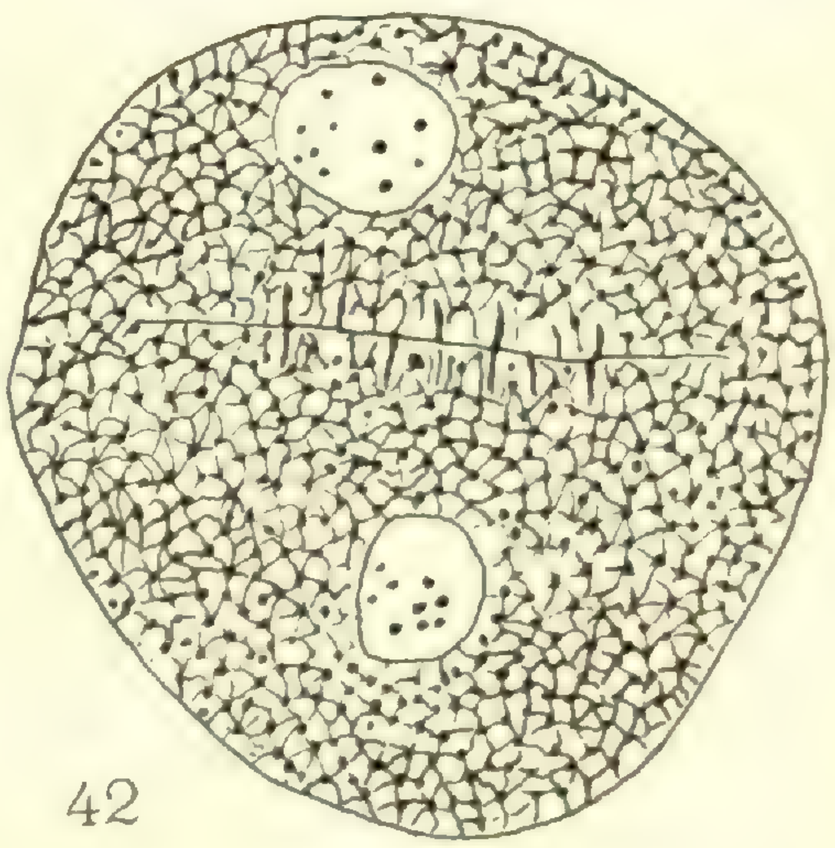

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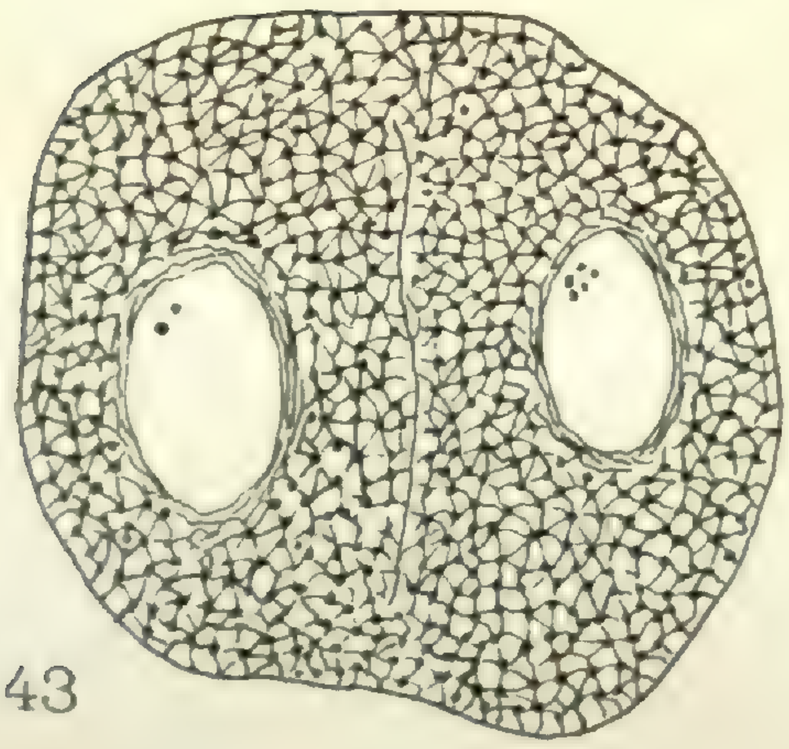

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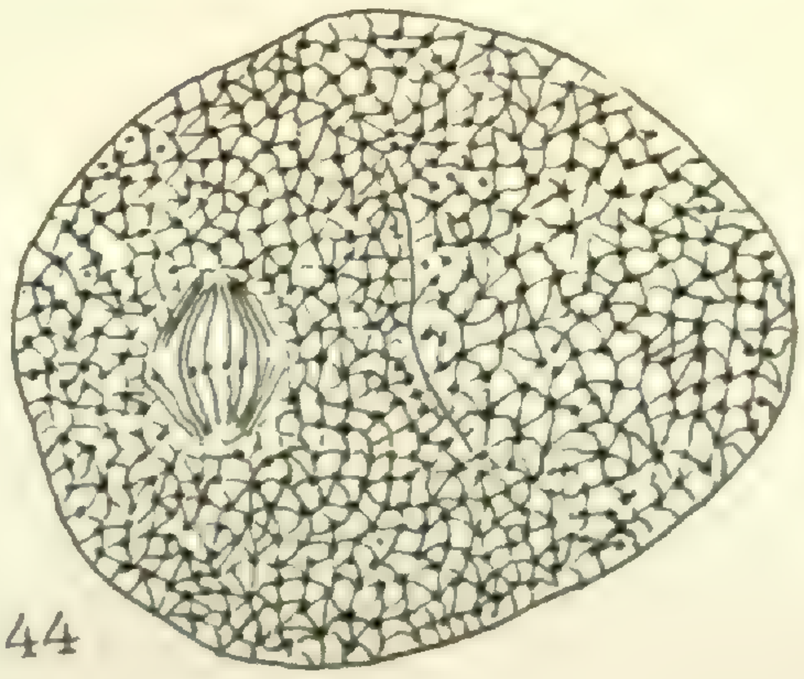

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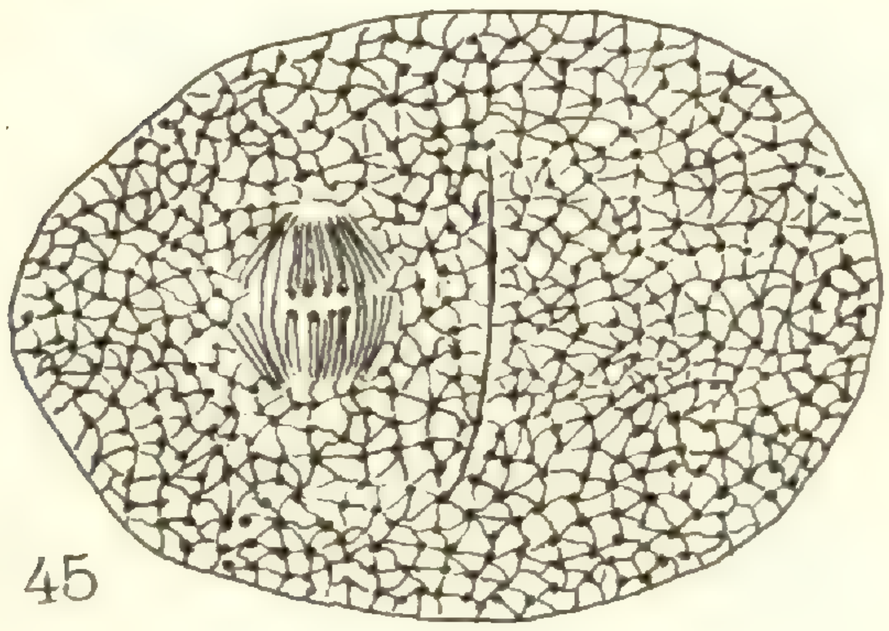

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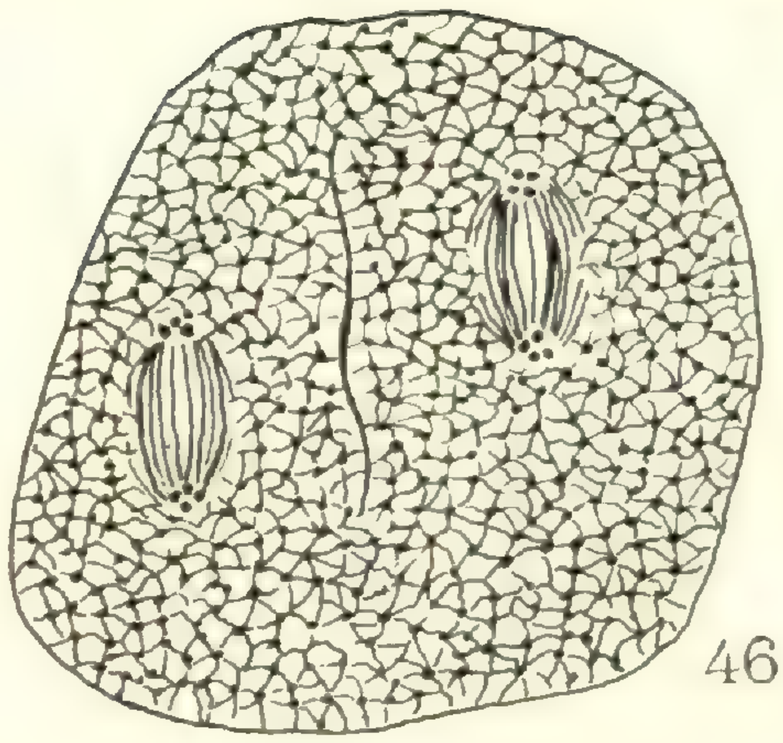

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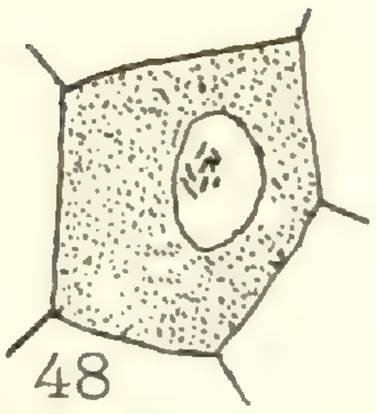

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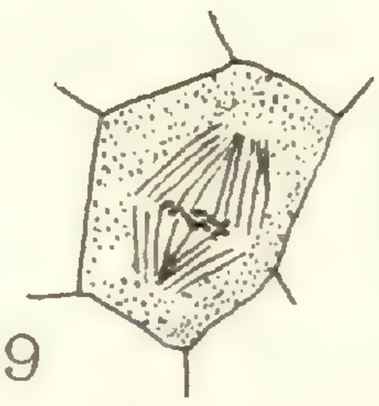

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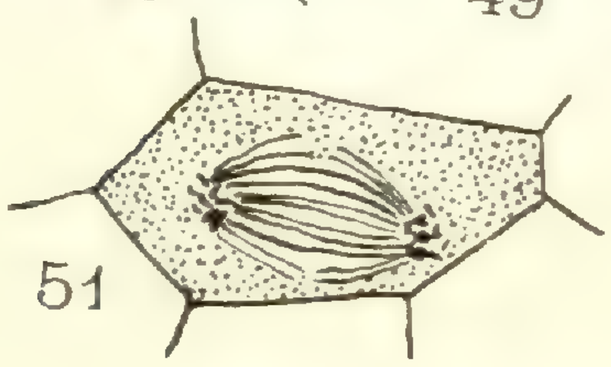

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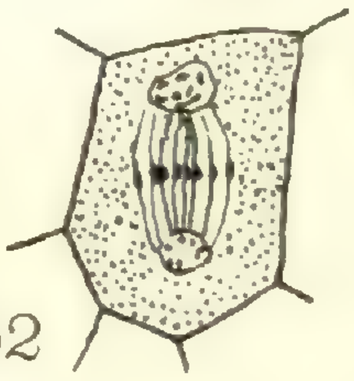

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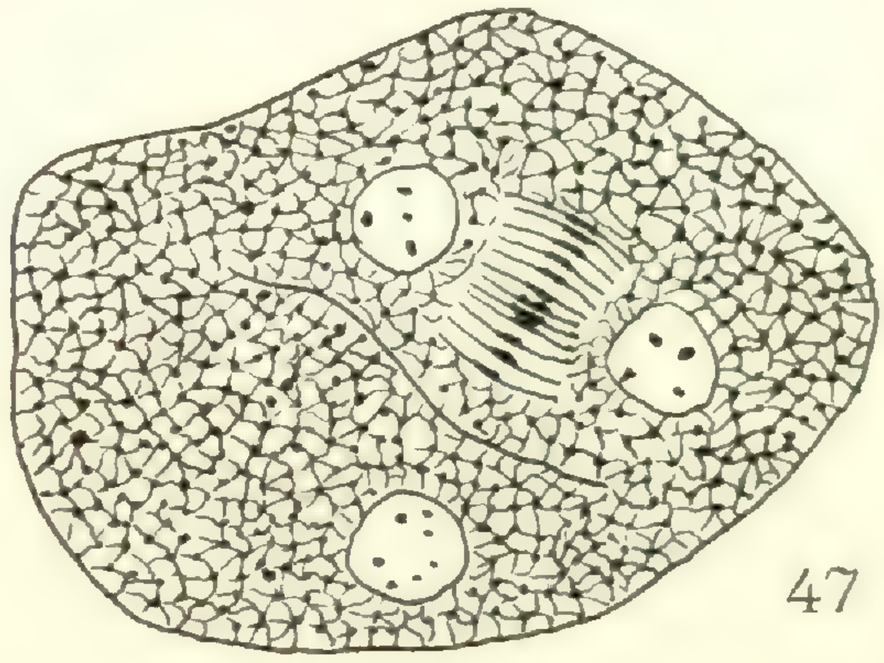

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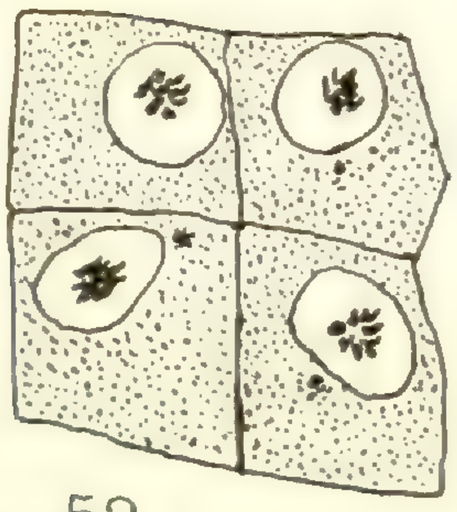

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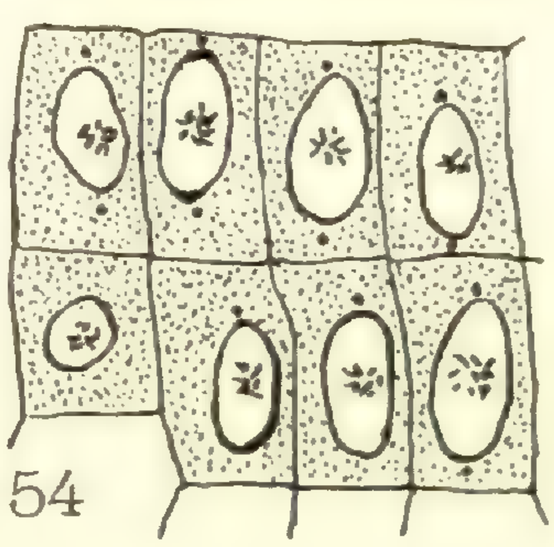

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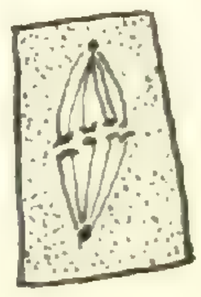

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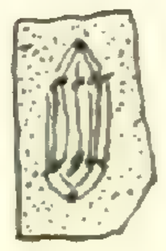

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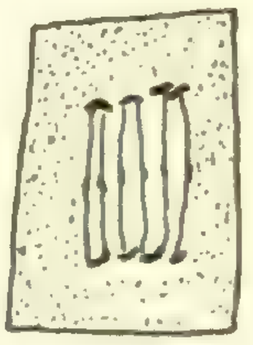

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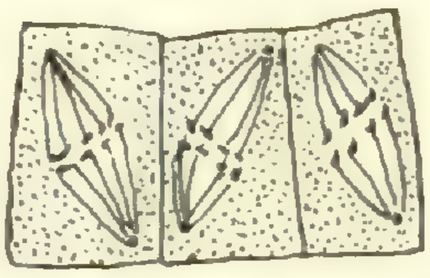

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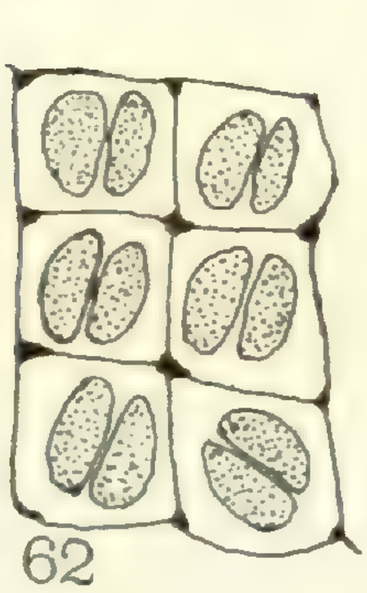

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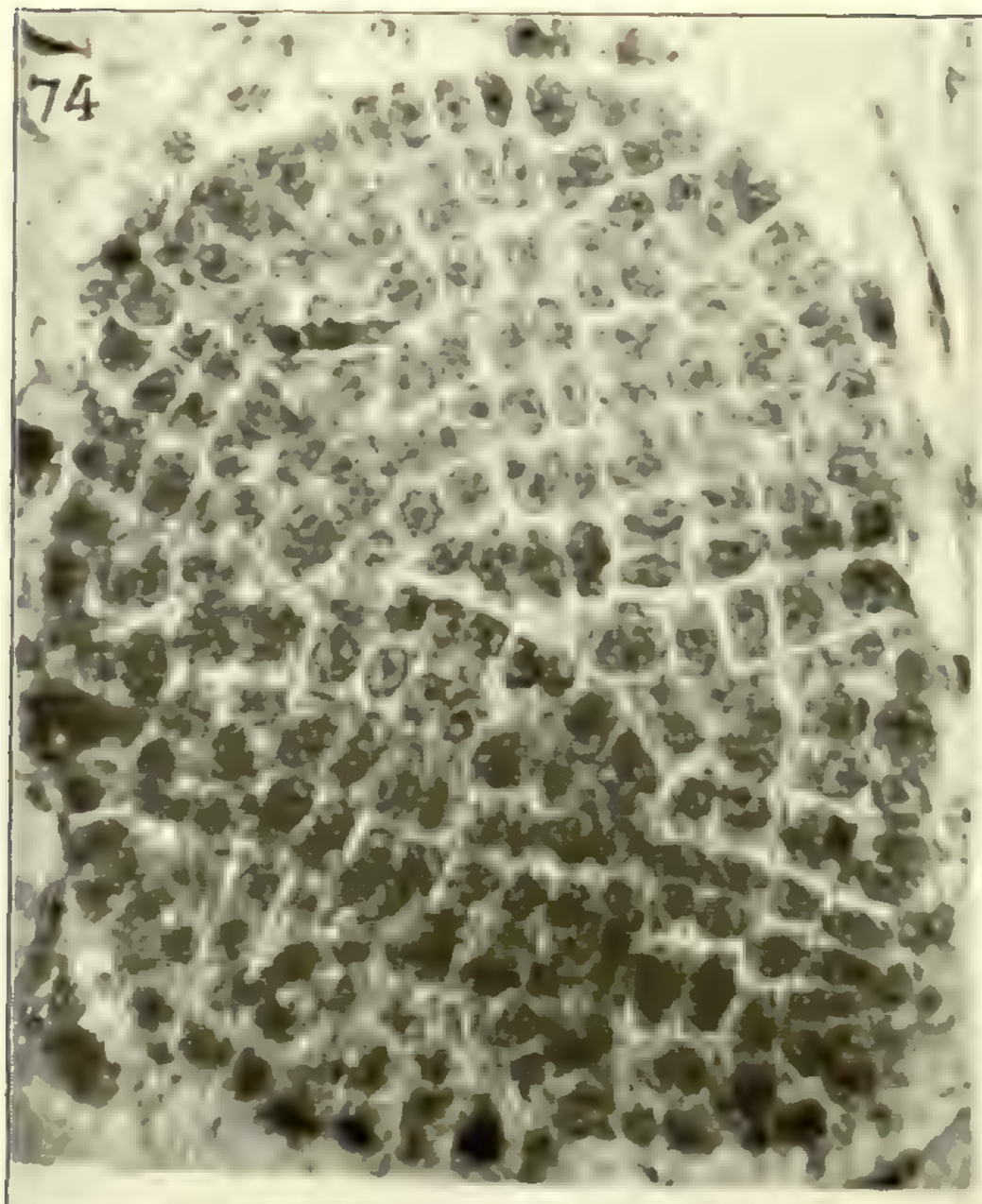

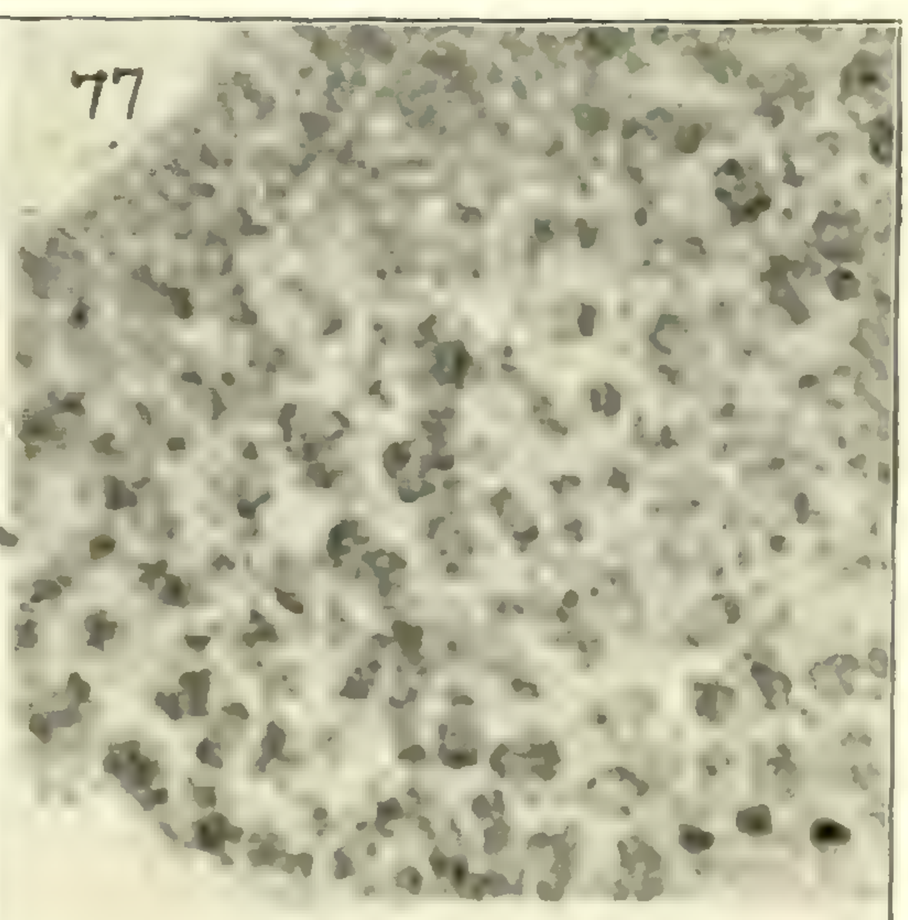

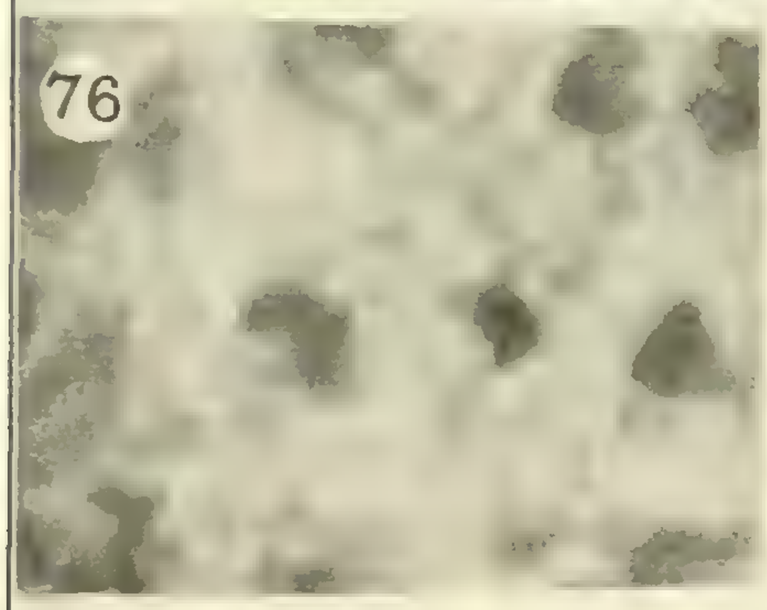

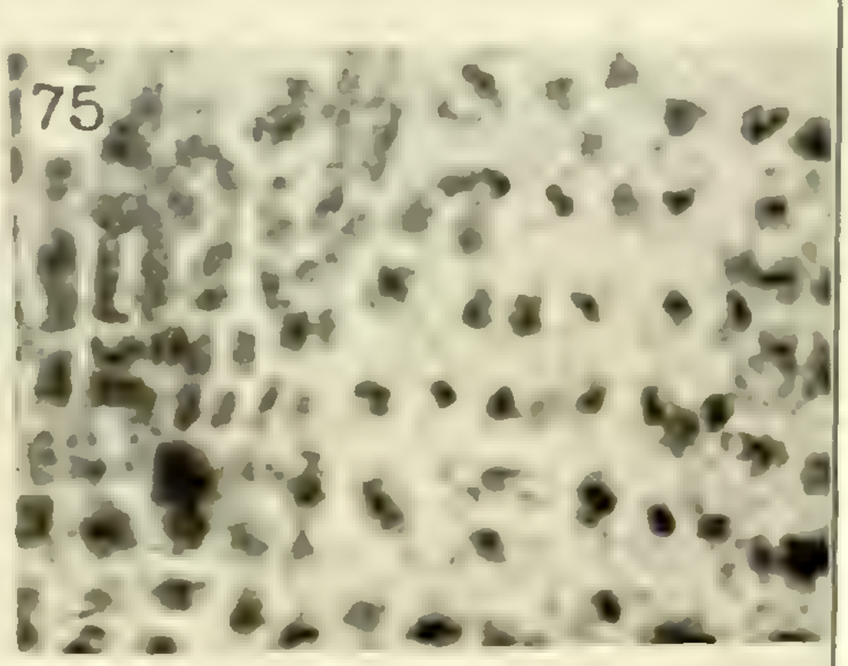

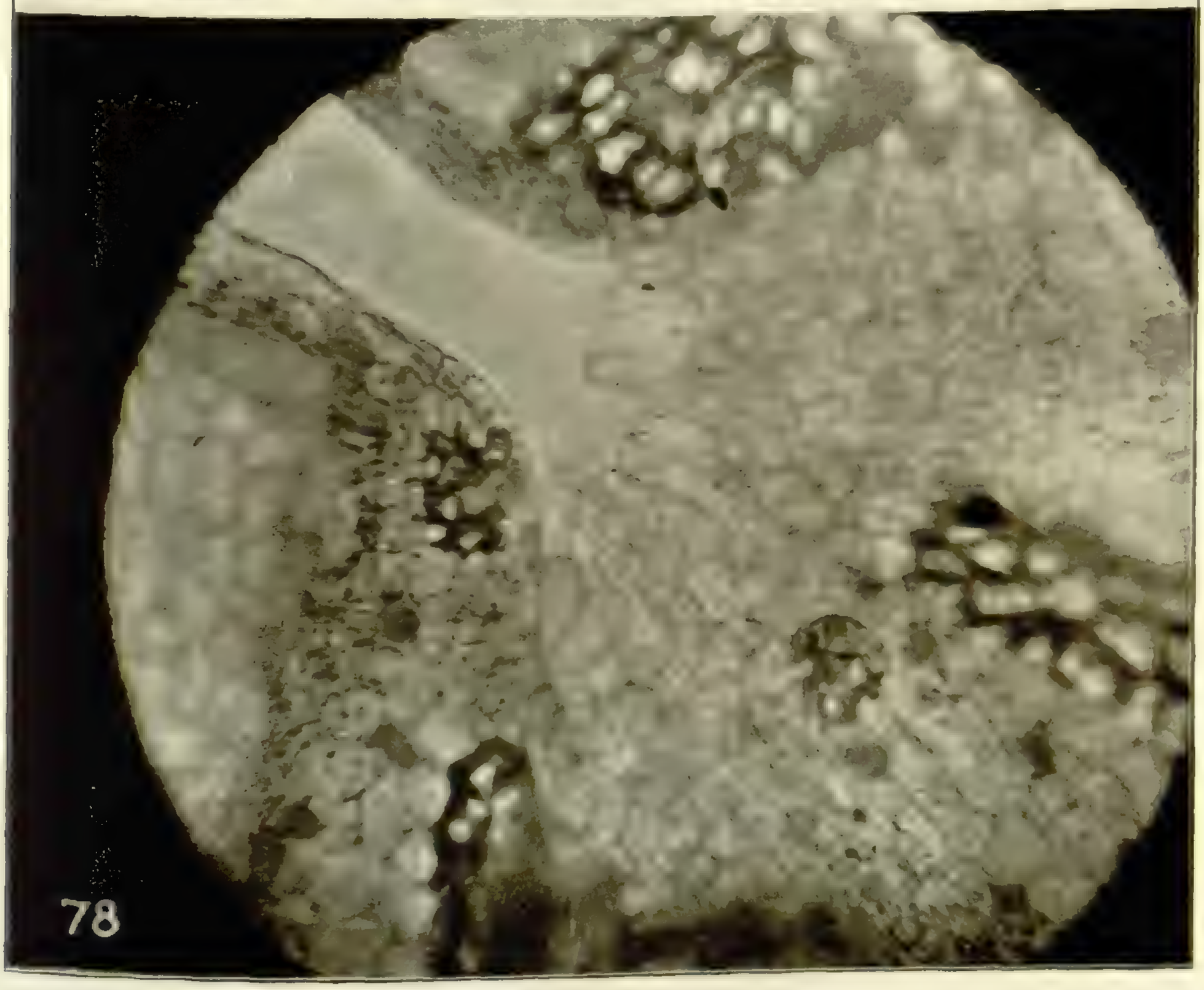

LEWIS on RICCIA

PLATE VII.

FIG. 34. Spore mother-cell in resting state. Chromatin on a fine linin network.

FIG. 35. The chromatin is in the form of an irregular thread.

FIG. 36. Chromosomes formed; weft of delicate fibres about the nucleus.

FIG. 37. Nucleus elongated and showing a weft of fibres.

FIG. 38. Spindle with chromosomes in plate. No centrosome.

FIG. 39. Chromosomes moving to poles of spindle.

FIG. 40. Chromosomes at the poles, thickening of spindle fibres to form cell plate.

FIG. 41. Daughter nuclei.

FIG. 42. Cell plate. Daughter nuclei contain numerous spherical bodies of chromatin which stain bright red with safranin.

FIG. 43. Daughter nuclei preparing for division.

FIG. 44. Daughter nucleus with chromosomes in plate. Neither centrosphere nor centrosome.

PLATE VIII.

FIG. 45. Chromosomes moving to poles.

FIG. 46. Daughter nuclei with chromosomes at the poles to form nuclei of spores. The cell plate formed in the first division persists.

FIG. 47. Second division completed.

FIGS. 48–52. Stages in the division of a sporophyte cell. No centrosome. *Fig. 48* shows slight radiation of cytoplasm from the poles of the elongated nucleus.

FIG. 53. Cells of antheridium which show a single rather irregular body near the nucleus.

FIG. 54. Cells of antheridium which shows the distinct centrosome-like bodies at the poles of the elongated nuclei. Compare *fig. 74*.

FIG. 55. Spindle with centrosome-like bodies at the poles.

FIG. 56. Centrosome-like bodies present when the chromosomes are almost at the poles.

FIG. 57. Cell from young antheridium. Chromosomes at poles. No centrosome can be distinguished.

FIG. 58. One cell preparing for last division, while the adjoining cell has the spindle formed and arranged diagonally.

FIG. 59. Diagonal arrangement of spindles in last division of cells in the antheridium.

FIG. 60. Curved spindles.

FIG. 61. Daughter nuclei formed after diagonal division; centrosome-like bodies present.

FIG. 62. Cells of antheridium after last division.

FIGS. 63–67. Spermatids in mother-cells.

FIGS. 68–73. Stages in the development of the spermatozoids.

PLATE IX.

FIG. 74. Antheridium in which the nuclei are elongated and preparing for division.

FIG. 75. Portion of section of an antheridium showing the spindles with the dense chromosomes in the plate and in some cases the centrosome-like bodies at the poles.

FIG. 76. Two cells of the same section enlarged three times.

FIG. 77. Chromosomes at the poles of the spindles.

FIG. 78. Cross-section of thallus showing the hyaline hairs which extend up into the median grooves.

BRIEFER ARTICLES.

NOTE ON THE RELATION BETWEEN GROWTH OF ROOTS AND OF TOPS IN WHEAT.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXXI.

STUDIES in the experimental morphology of plants have dealt mainly with the subaerial portions, comparatively little attention having been paid to variations in the growth of roots. Thus the literature of the subject is very meager. Several authors, especially MOELLER,[1] DETMER,[2] PERSEKE,[3] MER,[4] GAIN,[5] and FREIDENFELT,[6] have studied the relation of water to the growth of these organs, with the general result that in water itself they grow longer and thinner, with fewer branches and root hairs than are observed in moist soils. Curiously enough, in soils which are too dry for optimum growth, the response is very similar. The roots are long and slender and possess few branches excepting near their tips, which lie, of course, in moister soil.

The only observation on the relation of root growth to that of tops, with which the writer is acquainted, is that of MOELLER, to the effect that in a series of nutrient solutions, of concentrations ranging from 1.0 to 0.05 parts per thousand, the actual weight of roots produced varies generally with the concentration, but that the ratio of weight of roots to that of tops is much larger in the most dilute solution than in any of the others.

A number of experiments have suggested to the author that the accel-

[1]MOELLER, H., Beiträge zur Kenntniss der Verzwergung. Landw. Jahrb. **13**: 167-173. 1884.

[2]DETMER, W., Ueber den Einfluss äusserer Verhältnisse auf die Wurzelentwickelung. Landw. Versuchst. **15**: 107–113. 1872.

[3]PERSEKE, K., Ueber die Formveränderungen der Wurzel in Erde und Wasser. Leipzig. 1877.

[4]MER, E. De l'influence des milieux sur la structure des racines. Comptes Rend. **88**: 1277–1280. 1879.

———Recherches expérimentales sur les conditions de développement des poils radicaux. *Ibid*. 665–668.

———Des modifications de structure et de forme qu'éprouvent les racines suivant les milieux où elles végètent. Assoc. Franc. pour l'avance. sc. Compt. rend. de la 9e session. Rheims. 1880.

[5]GAIN, E., Rôle physiologique de l'eau dans la végétation. Paris. 1895.

[6]FREIDENFELT, F., Studien über die Wurzeln kraütiger Pflanzen. I. Ueber die Formbildung der Wurzel vom biologischen Gesichtspunkte. Flora **91**: 115–208. 1902.

erating or retarding effect of the soil upon plant growth may often be due primarily to a response of the roots themselves, and that the ordinarily observed effect upon the tops may be due to the nature of the roots rather than to that of the soil directly. This question deserves thorough study; the results to be given here cover only a very small portion of the field.

This work was carried on at the laboratories of the Bureau of Soils of the U. S. Department of Agriculture, Washington, D. C. The plant used was the Russian variety of wheat known as "Chul." The plants were grown directly from the seed in paraffined wire baskets of the form described by WHITNEY and CAMERON.[7] As these authors have already pointed out, such baskets possess the advantage over pots of producing a root system uniformly distributed throughout the soil mass, rather than the accumulation of roots on the inner surface of the vessel which occurs in the case of ordinary pots.

The studies to be discussed in this paper were made upon the roots developed in the first six series described in the author's previous publication[8] on the growth of tops, and in similar cultures. The medium used was a very poor soil from Takoma Park, Md., and the same soil with varying amounts of fermented stable manure added thereto.[9] The cultures of any series were placed side by side in a greenhouse, the amount of water in all the baskets being kept practically uniform by weighing at intervals of one or two days and adding the amount of water which was found to have been lost by transpiration. It is thus seen that the different cultures were all subjected to the same conditions excepting those which depend upon the treatment of the soil.

Series I of the paper on growth of tops[10] will serve as an example; the results of all the series are in accurate agreement. The soils and culture numbers were as follows:

Basket Number	1	2	3	4	5	6	7	8
Soil	Takoma Soil untreated	Do.+ 5000 p.p.m.[11] manure	Do.+ 10,000 p.p.m. manure	Do.+ 15,000 p.p.m. manure	Do.+ 20,000 p.p.m. manure	Do.+ 30,000 p.p.m. manure	Do.+ 40,000 p.p.m. manure	Do.+ 50,000 p.p.m. manure

[7] WHITNEY, M. and CAMERON, F. K., Investigations in soil fertility. U. S. Dept. Agric., Bureau of Soils, Bull. 23. 1904.

[8] LIVINGSTON, B. E., Relation of transpiration to growth in wheat. BOT. GAZETTE 40:1 78–195. *figs. 21*. 1905.

[9] For a description of this soil and a discussion of its properties, see LIVINGSTON, B. E., BRITTON, J. C., and REID, F. R., Studies on the properties of a sterile soil. U. S. Dept. Agric., Bureau of Soils, Bull. 28. 1905.

[10] A photograph of the tops and data for their growth are given in that paper.

[11] The abbreviation p. p. m. is used to denote parts per million by weight of air dry soil.

At the end of the experiment, which lasted seventeen days, the soil masses were taken from the baskets and the roots removed from them with as little injury to the latter as possible. The fresh roots thus prepared are shown in *fig. 1*. The numbers correspond to the culture numbers given above. It is at once evident that the root system increases in amount throughout the series. Closer observation shows that this is due mainly to differences in the relative number and length of secondary roots and succeeding branches; the primary roots are of the same number

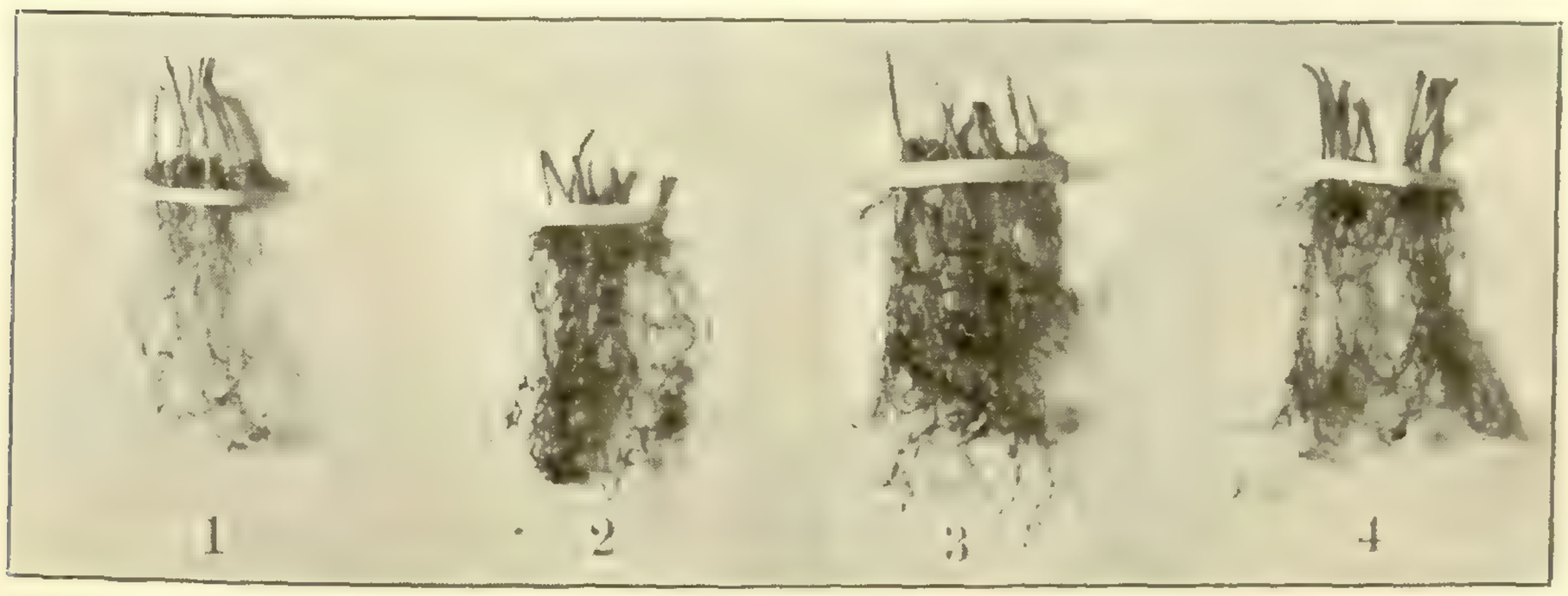

FIG. 1.—Roots from a series of wheat cultures grown in Takoma soil untreated and in the same with addition of 5000 p.p.m. to 50,000 p.p.m. of stable manure.

in culture 8 as in culture 1. They are somewhat longer and more slender in culture 8.

Photographs of single root systems from a similar series are shown in *figs. 2–5*, which bring out the last point more clearly than *fig. 1*. In *fig. 2*, which represents the roots from natural Takoma soil, very few branches are to be seen, and these are exceedingly short; practically the whole root system consists of the primary roots, with a few adventitious roots developed at the extreme base of the stem shortly after germination. *Fig. 3* shows roots from a soil containing 5000 parts per million of manure.

A very slight increase in number and length of branches is to be observed. *Fig. 4*, from a soil containing 10,000 parts per million of manure, shows numerous well-developed branches, while from a soil with 40,000 parts per million of manure, as shown in *fig. 5*, the branches have increased so markedly in number and extent as to make up by far the greater part of the system.

From these facts it is seen that, for this series of soils the variation in growth of tops is correlated with the number and length of lateral roots. The water content of all the soils was the same, so that the variations in

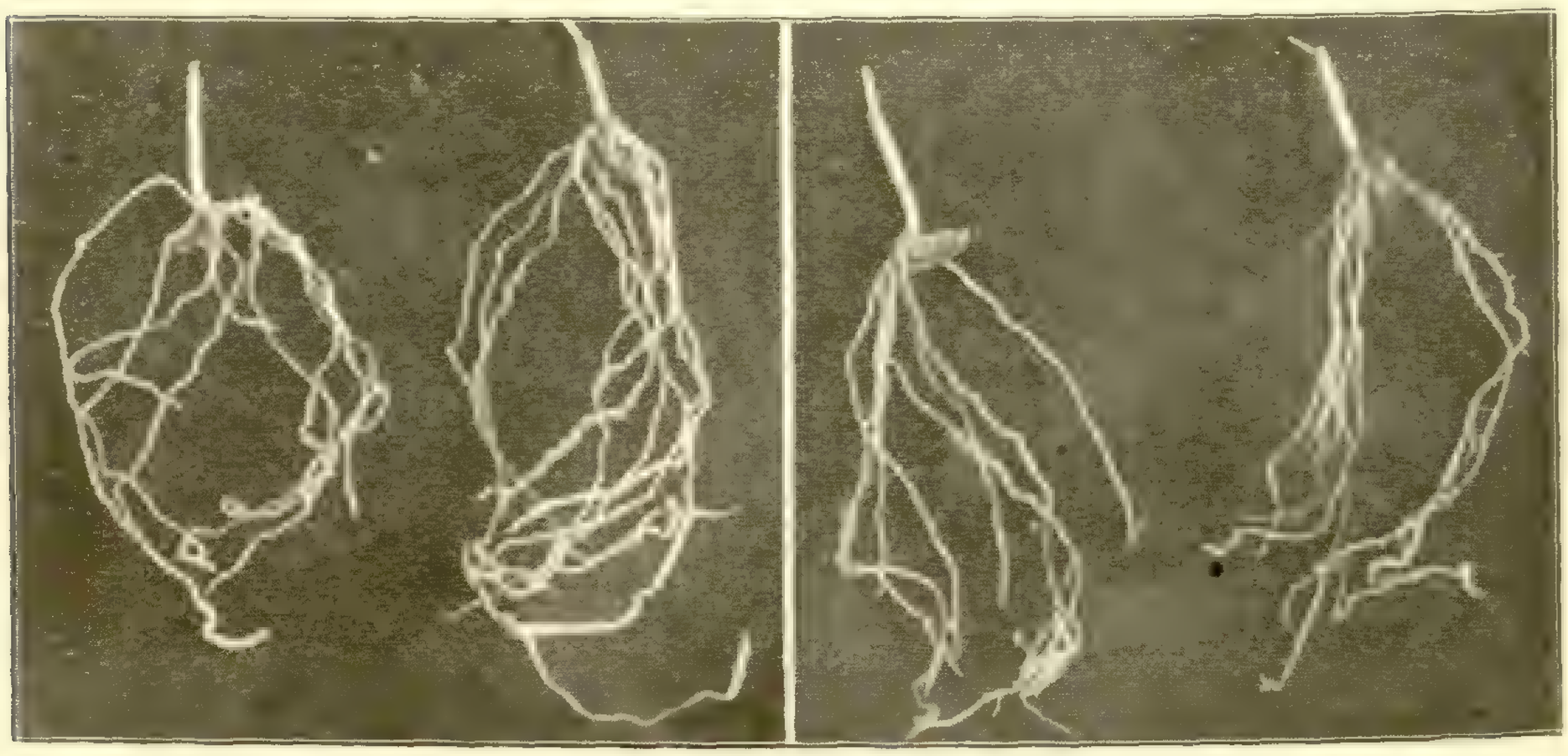

FIG. 2.—Wheat roots grown in Takoma soil untreated.

FIG. 3.—Roots grown in Takoma soil with 5000 p.p.m. manure.

growth cannot be related to this factor; therefore they must be connected with some unknown condition in the soil itself, a condition which is related to the amount of manure present.

The comparative anatomy of these roots was investigated, both by hand and paraffin sections, with the result that in the poor soils the main roots have a strong tendency to swell by direct enlargement of the cortical cells, without increase in the number of these cells, while in the better soils this tendency is not nearly so marked. Very old wheat roots from autumn stubble in the field show this balloon-like enlargement of the cortical cells to a still greater degree. This is apparently a phenomenon of age, suggesting that roots in the poor soils age more rapidly than in the better ones. It was also found that the zone of root hairs, which normally has its lower limit 5–10^{mm} from the root apex, extends in the poor soils to within 1–3^{mm} of the tip. The outgrowth of root hairs from the piliferous layer may also be related to the age of the cells; as is well known, these

organs normally appear only after the cells from which they arise have passed through their period of most rapid growth.

It would seem that the poor soil, by inhibiting branch growth and causing the enlargement of cortical cells, may render the root system unable to carry on an adequate amount of absorption for normal growth, and that this fact may be the main clue to an explanation of the stunted tops in such cases. That the inadequacy of the stunted roots is in regard to the water supply rather than to that of salts, is indicated by the fact that in distilled

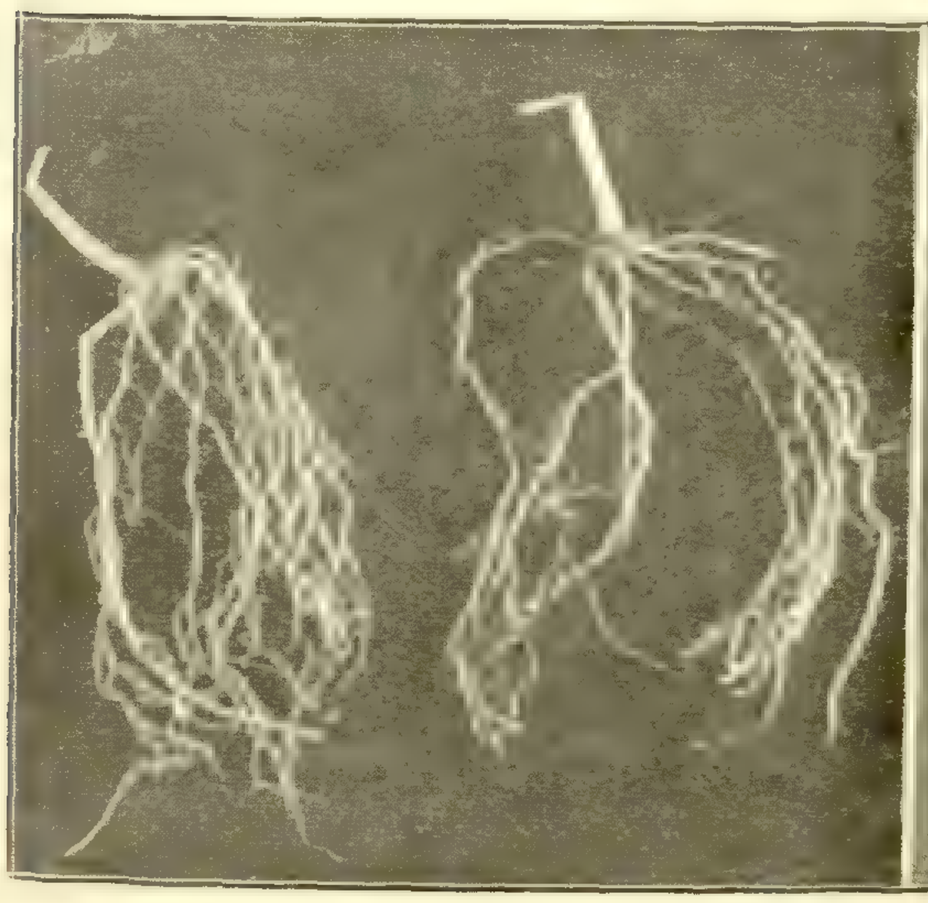

FIG. 4.—Roots grown in Takoma soil with 10,000 p.p.m. manure.

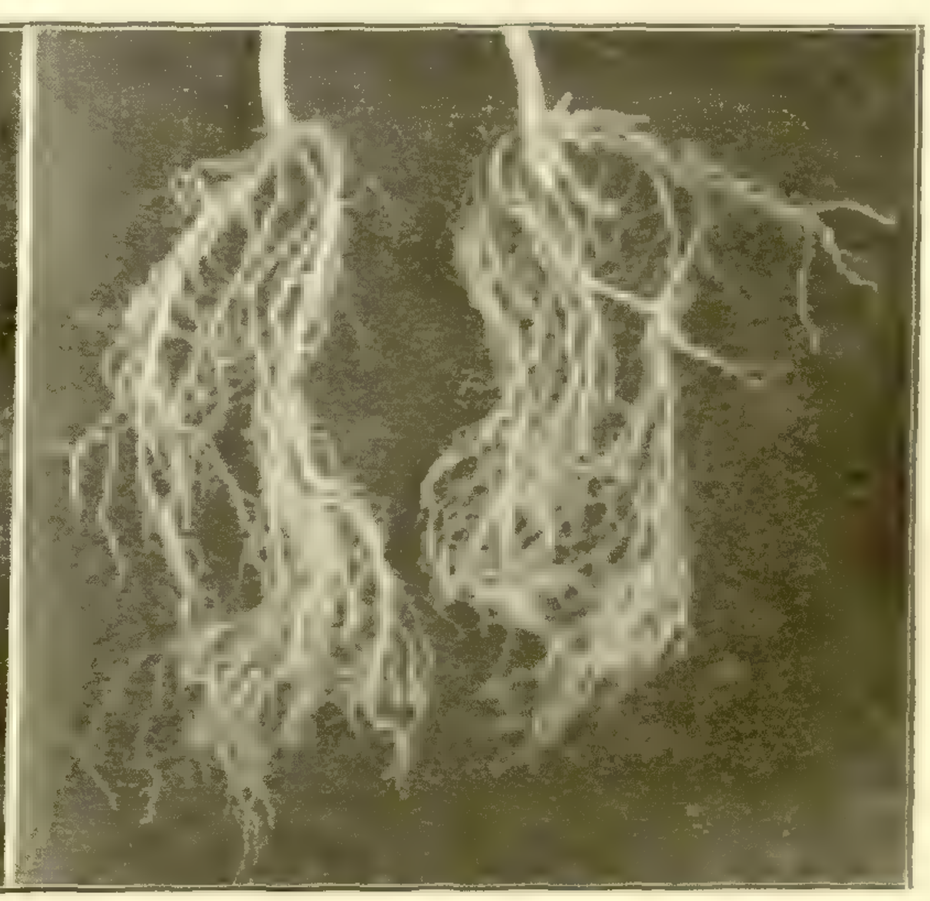

FIG. 5.—Roots grown in Takoma soil with 40,000 p.p.m. manure.

water, for the first two or three weeks, a better growth of tops is obtained than in the natural Takoma soil.

Determination of the relation of the dry weight of the root system to the nature of the soil was deemed advisable, but a number of tests yielded only negative results. The dry weight of the complete system was found to be practically the same from all of the soils. The variations were always irregular. This may, of course, be partially due to the fact that, while it is manifestly impossible to obtain anything like the entire root system of one of the better cultures (owing to the extreme fineness of the branches and their adhesion to the soil particles), yet from the poorer cultures, where branches are short and few, a much larger part of the system is obtainable.—BURTON EDWARD LIVINGSTON, *The University of Chicago, March 5, 1905.*

CURRENT LITERATURE.

BOOK REVIEWS.

The Swiss moors.

FRÜH and SCHRÖTER have published a remarkable work on the moors of Switzerland,[1] and have thus placed all who are interested in bog studies under lasting obligations. More than half of the huge volume is given over to the discussion of general geological, chemical, physical and biological problems connected with peat formation and the ecology of bog plants. One of the most valuable features is the discussion and correlation of the mass of European and foreign literature touching these problems.

After defining the scope of the investigation, the authors take up the peat-producing plant associations of Switzerland. These are described with the greatest detail, from the plankton to the arboreal vegetation; and their relation to peat accumulation is explained.

The moors are distinguished primarily as flat bogs, raised bogs, and bogs of the alpine regions. The first two types are of such general occurrence that their peculiarities may be briefly summarized here.

The *Flachmoor* is characterized as occurring in connection with waters rich in mineral matter, especially lime, in both wet and dry climates. Usually they show centripetal growth, and are dominated by species of Cyperaceae, Gramineae, Juncaceae and Hypneae. Species of Alnus, Betula, and Frangula make up the woody growth. Sphagnum, Ericaceae, and Empetrum are entirely wanting.

On the other hand, the *Hochmoor* type occurs under the influence of waters poor in mineral solutes; where the rainfall is abundant and the temperature mild or cold. The surface is convex. The oldest portion is toward the center; hence the growth is centrifugal. The dominant plants are Sphagnum, Oxycoccus, Andromeda, Calluna, Vaccinium, Empetrum, *Eriophorum vaginatum*, *Pinus montana uncinata*, *Betula pubescens*, and *B. nana*. These plants do not occur on the flat bogs, and are driven out by irrigation with waters of high mineral content, especially lime.

This summary suggests the essential difference between American bogs and the *Flachmoore* of European authors. Grass marshes occur here, which are strictly comparable. But the term "flat bog" is usually applied to areas

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[1] FRÜH, J. and SCHRÖTER, C., Die Moore der Schweiz mit Berücksichtigung der gesamten Moorfrage. Beiträge zur Geologie des Schweiz, herausgegeben von der Geol. Komm. der Schweiz. naturf. Gesells. Geotechnische Serie. III Lief. 4to, pp. xviii+751. Bern: A. Francke. 1904. *M* 40.

having a peat substratum, dominated by Sphagnum, Cassandra, Andromeda, Oxycoccus, Ledum, Vaccinium, etc.—a vegetation more nearly related floristically to the *Hochmoor*. Further, these occur in areas whose soil water is rich in mineral salts, frequently overlying marl! The raised bogs of America are strictly comparable to the *Hochmoor* of Europe, but their occurrence appears to be localized by climate rather than by the character of the soil water.

For both the flat and raised-bog plants, the authors conclude that the substratum is physiologically dry because of the combined influence of three factors: (1) high water-content of the substratum, (2) consequent low temperature and (3) the difficulties in the way of root respiration (due to wet soil and scarcity of oxygen), accompanied by a general impairing of all root functions.

The plants of the *Flachmoor* are noted for their ready absorption of mineral salts, absence of mycorhiza, unusual development of the underground parts, and the high percentage of ash. The cause of the xerophytic characters of many of the plants is not clear. They are probably connected with the difficulties of absorption. These plants are strictly hydrophilous, never occurring in dry situations.

The *Hochmoor* plants, however, absorb mineral salts with difficulty; mycorhiza occurs in all the Ericaceae, in Empetrum, Betula, and Pinus; carnivorous plants are common; the root systems are poorly developed; and the ash content is low. The xerophytic character of the plants is in part due to the difficulties of absorption, and in part to their evergreen habit. Many raised-bog plants also occur in dry situations.

The third and fourth chapters discuss the conditions and processes involved in peat accumulation, the chemical and physical properties of the end products, the classification of peats, the bog minerals, and the relation of bogs to coal deposits.

The geographic distribution of the bogs of Switzerland, a geomorphic classification of the moors of the world, the relation of settlement to moor development, the economics of the Swiss moors (with bibliography), the bog deposits as records of the postglacial history of northern Switzerland, form the other principal chapter headings of the first portion of the work.

The second division gives a detailed description of the individual Swiss bogs. In most instances these include not only plant associations of the present surface but also the succession of plant remains occurring in the peat. The location of the bogs is made clear by an excellent topographic map, upon which the distribution of the several bog types is shown. The bibliography occupies seventeen pages and includes only the more important purely scientific papers.

The importance of the work from the standpoint of future investigation is undoubted. To American students it furnishes not only a key to the present status of the subject, but also a model for the study and description of our own bogs, marshes, and swamps.—E. N. TRANSEAU.

Reproduction of mildews.

HARPER has brought together the results of several years of study of nuclear activities in the mildews in a lengthy and beautifully illustrated publication from the Carnegie Institution.[2] It is impossible for us to consider more than the striking new features of his investigations. The paper contains a résumé of much of his earlier work and a broad discussion of many cytological principles which are of general interest and will richly repay the reader of this very creditable contribution to American botany. The author takes a strong stand for critical morphological analysis and classification of the stages in the life history of thallophytes, with a clear separation of phylogenetic history from physiological functions.

The most important new features of HARPER'S research, chiefly in Phyllactinia, are (1) the establishment of a "central body" within the nucleus, which constitutes a point of attachment for the chromatic elements and gives a clear polarity to the structure, and its continuous existence through the most important phases in the life history; (2) the evidence for the permanence of the chromosomes; and (3) the evidence that the triple mitoses preceded by synapsis in the ascus constitute a double reduction of the chromosomes which are quadrupled by the two nuclear fusions in the life history, the first fusion at the time of the sexual act and the second fusion within the young ascus.

The central body is a permanent structure, always present in the resting nucleus, dividing with each mitosis, and the center for an arrangement of chromatic threads within the nucleus and for the attachment of spindle fibers during nuclear division. Its position determines a pole in the nucleus around which are grouped the chromatic elements, which are thus always in connection with the central body, both in the resting nucleus and during mitosis. This constitutes new evidence for the permanence of the chromosomes throughout the succession of mitoses in the life-history. HARPER has not been able to distinguish the different sets of chromosomes after the nuclear fusions, for the chromatic elements and the central bodies unite very intimately. But the second fusion in the life history, that in the ascus, is followed at once by a period of synapsis and the triple mitoses out of which come the eight chromosomes characteristic of the gametophytic phase of the form.—B. M. DAVIS.

MINOR NOTICES.

Observations in Spitzbergen.—The flora of Spitzbergen is fairly known. Therefore, DR. WULFF, who accompanied the Swedish expedition for the measurement of an arc of the meridian, undertook to make ecological observations on the arctic plants,[3] especially touching their transpiration, occurrence of mycorhiza

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[2] HARPER, R. A., Sexual reproduction and the organization of the nucleus in certain mildews. Imp. 8vo. pp. 104. *pls.* 7. Washington: Carnegie Institution of Washington. 1905.

[3] WULFF, THORILD, Observations botaniques faites au Spitzberg. Missions scientifique pour la mesure d'un arc de méridien au Spitzberg. Mission Suédoise. Tome II, X^e^ section, Botanique. Traduit de l'Allemand par H. MARCEL HARDY à Dundee. 4to. pp. 63, *pls.* 4. Stockholm. 1903.

and anthocyan, the vegetation of the "polygonal" soils, and to make miscellaneous floristic notes at various stations. The transpiration he finds very feeble and almost without diurnal periodicity or plant control. This feeble transpiration he accuses of being a *cause* of feeble growth; instead, is not its feebleness due to the same cause as the feebleness of growth, the low supply of energy? Mycorhizas, internal and external, are common. Anthocyan is found in fifty species, about half the known higher plants. It is always lacking in plants growing on soil enriched by the droppings of wild birds, whereas the same species growing on poor soils show it abundantly. As to the rôle of anthocyan, he holds it for an absorber of energy, and without it no plant can become dominant in arctic regions. For other interesting observations one must consult the work itself.—C. R. B.

Polypodiaceæ and edible fungi.—Not that there is any connection between them; but both are treated by COPELAND in a bulletin[4] from the Government Laboratories at Manila. The section on Polypodiaceæ forms the bulk of the bulletin and is "an attempt to collect and publish descriptions of all the ferns known to have been found in these islands." The author adds: "I am not personally acquainted with a large part of those ferns still known here only from earlier collections." Which leads us to remark that he should then have abstained from describing a new genus and new species among them, as he did in DR. PERKINS's last *Fragmenta*. In reprinting here these descriptions he has neglected to indicate that they have already been published elsewhere. He has sinned again in adding one more new name in this bulletin. The compilation of such descriptive floras is undoubtedly serviceable; but one who is not a taxonomist and who confesses the absence of indispensable books and specimens, should not take the chances of cumbering pteridology with new names which may or may not be justified. And the same may be said regarding the brief fungus part.—C. R. B.

Genera of Mexican plants.—The flora of Mexico is so closely related to our own that any work on it is of essential assistance to American taxonomists. So we welcome the assembling and description of the Mexican genera, and the listing of the species, undertaken by Professor CONZATTI, director of the State Normal School of Oaxaca, of which the first volume, on Polypetalae, has recently been published by the Ministry of Public Works. This volume[5] begins with an artificial key covering about 50 pages including all genera, and contains descriptions of 667 genera of Polypetalæ, representing 71 families, and including close to 4,500 species. This is to be followed by another on Gamopetalae and a third

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[4]COPELAND, E. B., I. The Polypodiaceæ of the Philippine Islands. II. New species of edible Philippine fungi. Bureau of Government Labs. Bull. 28. 8vo. pp. 146. *pls.* 3. 1905.

[5]CONZATTI, C., Los géneros vegetales mexicanos. Imp. 8vo. pp. 449. Mexico: Oficina Tip. de la Secretaría de Fomento. 1905. $3 (Mexican).

on Monochlamydeae, Monocotyledoneae, Gymnospermeae, and Pteridophyta, embracing in all about 1900 genera.

The descriptions are very full, and though the diagnostic characters are not indicated, this is largely atoned for by the complete system of synoptic characters under the tribes and subtribes.—C. R. B.

Germs of mind in plants.—A little book,[6] unknown to us in the original French, now translated into English by A. M. SIMONS, well-known for his work in Chicago along social and philanthropic lines, shows that there exists in France the same sort of popularizers of science as in our country—writers who with a smattering of scientific knowledge lack the fuller knowledge that forms a background and furnishes scientific perspective. The facts of plant ecology are herein so distorted in their relation as to become caricatures; the use of words is so fanciful as to convert sober ideas into grotesque fairy-tales. For this, doubtless, the author is chiefly responsible; but the translator slips occasionally through unfamiliarity with a technical use of some common word.

The book is interesting; but it is as little "science" as a historical novel is history. It is difficult to see how such fiction can be "a contribution to the cause of socialism and science."—C. R. B.

Hepaticae of France.—LACOUTURE has prepared a helpful series of descriptive analytical keys to facilitate the identification of French liverworts by amateurs.[7] The keys are arranged in a convenient bracket fashion, which is easy to use but makes the form of the thin volume rather unhandy and precludes its use as a field manual. The description of each species is accompanied by an excellent figure illustrating the most essential features described. The keys, in the form of tables, are arranged in three series, of which the first, consisting of tables I and II gives the characters of the tribes; the second, tables III–IX the characters of the genera; and the third, tables XII–XXXIX, the characters of the species and the illustrations. No attempt is made to exhibit the natural classification.—C. J. CHAMBERLAIN.

Index Filicum.—The fourth and fifth fascicles of CHRISTENSEN's important work[8] were issued respectively in October and December last. They carry the references from *Cyathea lanuginosa* to *Gleichenia cryptocarpa*. The huge genus Dryopteris alone takes fifty-two pages, which indicates something of the comprehensiveness of the work. Let colleges and libraries hasten to support by their

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[6]FRANCÉ, R. H., Germs of mind in plants. Trans. by A. M. SIMONS. 12mo. pp. 151. Chicago: C. H. Kerr & Co., 1905. 50 cts.

[7]LACOUTURE, CH., Hépatiques de la France. Tableaux synoptiques de. caractères saillants des tribus, des genres, et des espèces. 4to. pp. 78. *figs.* 200. Paris: Paul Klincksieck. 1905. *fr.* 10.

[8]CHRISTENSEN, C., Index Filicum, etc. Fasc. 4, 5. Copenhagen: H. Hagerups Boghandel. 1905. Each 3*sh.* 6*d.*

orders the stupendous and too thankless task which the author has undertaken. The employment of the American system of citation is notable.—C. R. B.

Das Pflanzenreich.[9]—Of this work parts 22 and 23 have lately appeared, including respectively the Primulaceae by PAX and KNUTH, and the Halorrhagaceae by SCHINDLER. The rate at which these monographs are appearing is remarkable, and shows something of the energy of the editor and his sagacity in the selection of his collaborators. The publisher's part, too, is admirably done.—C. R. B.

Eucalyptus.— MAIDEN'S revision[10] has now reached part 7, which includes *EE. regnans, vitellina, vitrea, dives, Andrewsi,* and *diversifolia*, and is illustrated by four plates.—C. R. B.

NOTES FOR STUDENTS.

Items of taxonomic interest.—ZAHLBRÜCKNER lists (Beihefte Bot. Cent. **19**²: 75–84. 1905) the lichens collected by Professor D. H. MEYER in the Ecuador highlands in 1903, describing six new species.—CARDOT (*idem* 85–148. *figs. 39*) enumerates 125 species of the mosses of Formosa, collected by Abbé FAURIE in 1903, bringing the total known species of this island to 130, of which 39 are new. *Herpetineuron* (C. Müll. as Anomodon §) is raised to generic rank.—ENGLER describes (Bot. Jahrb. Syst. **37**: 95, 96. 1905) a new genus of Araceae, *Ulearum*, and in his tenth contribution to a knowledge of the Araceae, (*idem*) adds to the family nearly a hundred new species, chiefly from Central America, the subequatorial andine province, the Philippines, and East Indies.—DIETEL, in his sixth paper on Japanese Uredineae (*idem* 97–109) describes 16 new species, and in one on Japanese fungi (*idem* 156–160) ten others.—RADLKOFER (*idem* 144–155) describes 8 new species of Serjania and 8 of Paullinia (Sapindaceae) from Peru, Brazil, Bolivia, and Columbia.—STEPHANI (Bull. Herb. Boiss. II. **5**: 885–900, 917–946. 1905) in his *Species Hepaticarum* concludes the treatment of the genus Plagiochila, describing 26 new species, a number of them from equatorial America.—DOMIN (*idem* 947, 948) describes 2 new species of Koeleria from Asia, and BEAUVERD (*idem* 948) a new Burmannia from Brazil and (990–991) a new Hesperantha from the Transvaal.—FERNALD characterizes (Ottawa Nat. **19**: 156. 1905) a new variety of *Antennaria neodioica* Green from E. Quebec.—SCHNEIDER, in a prodromus to a monograph of Berberis (Bull. Herb. Boiss. II. **5**: 139 ff. 1905) recognizes 159 species, among them a number of new ones of his own creation, which he divides into 21 sections. The regions of their

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[9] ENGLER, A., Das Pflanzenreich. Heft 22, Primulaceae by F. PAX and R. KNUTH. pp. 386, *figs. 75 (311), maps 2. M* 19. 20.—Heft 23. Halorrhagaceae by ANTON K. SCHINDLER. pp. 133, *figs. 36 (196). M* 6. 80. Leipzig: Wilhelm Engelmann. 1905.

[10] MAIDEN, J. H., A critical revision of the genus Eucalyptus. 4to. pp. 183–205, *pls. 33–36.* Sydney: Government N. S. Wales. 1905. *2sh. 6d.*

dominance are South America and E. Asia.—HELLER describes (Muhlenb. 1: 124) a new Veratrum from Idaho, and (*idem* 125) a Linanthus or Gilia from California.—MCALPINE adds a new genus, *Uromycladium*, to the Uredineae (Ann. Mycol. 3: 303–323. *pls.* 6–9. 1905). It is based on 7 Australian species occurring on Acacias, and is intermediate between Uromyces and Ravenelia.—VUILLEMIN shows the identity of *Hartigiella* with *Meria* (*idem* 340–343).—SCHMIDLE found in plankton material from Roxheim, Bavaria, a new alga, for which (Hedw. 45: 34. 1905) he erects the genus *Didymogenes*.—FLEISCHER (*idem* 53 ff.) proposes an extension of the moss family Pterobryaceae to include five other families, in whole or in part, and gathers from various genera some 25 species to swell his genus Pterobryopsis. He establishes a new monotypic genus *Müllerobryum* on an Australian moss already referred to 3 separate genera. *Trachypodaceae* is a new family, and *Trachypodopsis* its characteristic new genus, for both of which he has "gathered of every kind," and Teil I is only begun!—PECK (Rept. N. Y. State Botanist 1904) describes new fungi; Boletus (3), Clavaria (2), Cortinarius, Lactarius (2), Pholiota.—CARDOT finds 35 new species of mosses in SKOTTSBERG'S collections made on the Swedish antarctic expedition (Bull. Herb. Boiss. II. 5: 997–1011. 1905).—HIERONYMUS has studied (Bot. Jahrb. Syst. 36: 458–573. 1905) the Compositae collected by JELSKI in Peru, among which he finds 58 new species.—DIELS (*idem* Beiblatt 82: 1–138) makes hundreds of additions to his flora of central China, including many new species and three new genera, *Giraldiella* Dammer (Liliaceae), *Pteroxygonum* Dammer and Diels (Polygonaceae), and *Biondia* Schlechter (Asclepiadaceae).—NELSON describes (Proc. Biol. Soc. Wash. 18: 171–776. 1905) new species from Nevada in Cleomella (2), Sphaerostigma, Zauschneria, Rhamnus, Polemonium, Artemisia, and a new genus of Solanaceae, *Bosleria*.—RENAULD and CARDOT in their tenth paper on *Musci Exotici* (Bull. Soc. Roy. Bot. Belgique 41: 7–122. 1905) describe, among many others, largely Mascarene and East Indian, 9 new species from Porto Rico, 3 from Costa Rico, one from Guadeloupe, 3 from Cocos Island (Pacific Cent. Am.), 1 from Mexico, and 1 from Hawaii. They also establish as a new genus of Hypnaceae Müller's section of Hypnum, *Dimorphella*. The same authors (*idem* 123 ff.) in their third article on *Musci Costaricenses* describe 22 new species.—HELLER has found some new species in his collections for 1905 in California and describes them (Muhlenb. 2: 1–6. 1905), under Eriogonum (3), Montia, Delphinium, Ranunculus, Thysanocarpus (2), Lithophragma, Ribes, and Amelanchier.—HOWE adds several algae to our flora (Bull. Torr. Bot. Club 32: 563–586. *pls.* 23–29. 1905) from the Bahama-Florida region; Halimeda, Avrainvillea, Sarcomenia, Dudresnaya, and a new genus *Cladocephalus* (Codiaceae), besides changing several names.—UNDERWOOD (*idem* 587–596) maintains the genus *Alcicornium* Gaud. as valid, gives a synopsis of the species, and describes *A. Veitchii* as a new species.—RYDBERG, about to publish a Flora of Colorado, makes (*idem* 597–610) what he considers necessary changes in names, and describes new species of Deschampsia, Eatonia, Poa (9), Festuca (2), and

Elymus (2).—OSTERHOUT proposes from Colorado (*idem* 611–613) new species of Allionia, Aster, Senecio, and Carduus (2), which are respectfully referred to Mr. RYDBERG.—SARGENT adds (Rhodora 7: 192–219. 1905) 24 new species of Crataegus, all from New England.—ROBINSON describes (*idem* 219–222) a new Ranunculus from Gaspé and Labrador.—C. R. B.

Fossil gymnosperms.—Two trunks of Cycadoidea have been found in the Portland beds of Boulogne, to which MM. FLICHE and ZEILLER give the specific name *C. pumila* on account of their small size.[11] Another Cycadoidea is described without attribution of a specific name. An interesting and important discovery is a cone of Sequoia of the *S. gigantea* type, which is named *S. portlandica*. The oldest well authenticated cone of Sequoia previously known is Heer's *S. lusitanica* from the Wealden beds of Portugal, which belongs to the type represented by the living *S. sempervirens*. It thus is demonstrated that Sequoia existed in its two living types as far back as the Jurassic period and must thus be very much more ancient in its first appearance. Other important discoveries are pine-cones representing the two main series of the present day, viz., the sections Strobus and Pinaster. The cone of the Strobus type is very much flattened and does not yield any definite information as to its internal organization, so the authors include it under the provisional fossil genus Pinites, with the specific appellation *P. strobiformis*, which would appear to be too close to our western *Pinus strobiformis* to stand as a permanent name. The other cone is exceedingly well preserved and resembles very closely, as the authors point out, small cones of the living *P. Laricio*. This cone is referred to Pinus as *P. Sauvagei*. These observations are of very special interest because they establish that Pinus too must be a very old genus, since examples of both the hard and soft pine series existed already in the Jurassic.

GOTHAN calls attention to the somewhat unsatisfactory condition of *Xylopalaeontolgie* at the present time and by comprehensive study of fossil and living woods, including many type-specimens of the former, reaches a number of conclusions of greater or less importance.[12] The proposition of FELIX to divide fossil woods presenting tracheary structure resembling that of living Araucarineae, into Cordaioxyla for the palaeozoic woods, which may be supposed to be those of Cordaites, and into Araucarioxyla for mesozoic and later woods, is rejected, since in the author's opinion no distinction can be made histologically between the two. For these woods ENDLICHER'S name *Dadoxylon* is retained. *Cedroxylon* Kraus and *Cupressinoxylon* Goeppert are separated from each other, not on the basis of the presence of resiniferous parenchyma in the latter genus and ts absence in the former, but on the character of the medullary ray-cells, since many Cedroxyla and even Pityoxyla have resinous parenchyma. This distinction has

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[11] FLICHE, P., et ZEILLER, R., Note sur un florule portlandienne des environs de Boulogne-sur-Mer. Bull. Soc. Geol. de la France IV. 4: 787–812. 1904.

[12] GOTHAN, W., Zur Anatomie lebender und fossiler Gymnospermenhölzer. Abhandl. k. preuss. geol. Landesanstalt, Neue Folge, Heft 44. 1905.

already and previously been clearly made by PENHALLOW. The author also attempts to separate the woods of the Podocarpeæ from those of the Cupressineae in the larger sense, on the basis of the structure of the pits in the ray-cells. The success of this distinction may be judged from the fact that it results in putting Sciadopitys with the Podocarpeae. *Pityoxylon* of Kraus is broken up by this writer into two genera, *Piceoxylon* and *Pinusoxylon*. The latter genus represents the wood of Pinus, and seems somewhat unfortunate, since it is doubtful if the mesozoic pines had the wood structure which is found as characteristic of that genus in Tertiary and modern times. There are also disquisitions on spiral striation in the wood of the gymnosperms and on the value of annual woody rings as diagnostic of geologic formations. The work closes with two tables for the determination respectively of living and fossil gymnospermous woods. There is likewise an index and an alphabetical list of the living woods investigated by the author.—E. C. JEFFREY.

Injury by smoke.—Frequent controversies and law suits, arising from damage to agricultural crops by the smoke produced by manufacturing establishments in Germany, have made the recognition of this form of injury extremely important. In order to furnish a basis for distinguishing smoke-injury from injuries due to other factors, SORAUER[13] has made a comparative anatomical study of various kinds of injury commonly occurring in the more important grains, oats, wheat, and barley. The paper contains detailed comparative descriptions of changes in the cell walls and cell contents which cannot be severally noted here. The general plan followed in each case is represented by the following heads: The behavior of the normal plant in its gradual, natural dissolution; abnormalities in smoke-free regions; the phenomena in plants injured by chlorin and by hydrochloric acid fumes; experimental tests of the influence of hydrochloric acid fumes; phenomena confused with smoke injuries. In natural death the cells lose a large part of their contents and finally (except the epidermal cells) collapse completely. This process first involves the tip and edges of the leaves. In cases of death resulting from other causes, as drought, the cells do not collapse so completely, since the contents are not fully resorbed. In injuries due to acid fumes from smoke, the contents of the mesophyll cells contract into an irregular greenish lump, while the cell walls partially collapse.

The most striking feature about this form of injury is the collapse of the epidermal cells. The accompanying changes of the cell contents and cell walls in these and in many other forms of injury are minutely described. The recognition of smoke injury in general is based on the fact that the cells, dying rapidly, collapse partially without being emptied of their contents, the epidermal cells showing the same phenomena. The author continually emphasizes the fact, however, that no clearly defined symptoms for the absolute and certain recognition of smoke injury can be given, but that in all cases a comparative study of

[13] SORAUER, P., Beitrag zur anatomischen Analyse rauchbeschädigter Pflanzen. Landw. Jahrb. **33**:585–664. *pl. 15–18.* 1904.

plants growing under the immediate influence of the acid fumes and others growing under similar conditions but not within the smoke zone, must be made. —H. HASSELBRING.

Viticulture.—Recent publications from the Royal Hungarian Central Institute of Viticulture are as follows: Volume III, part 2, consists of chemical analyses of the stems and shoots of American species used for stocks in Hungary.[14] The points determined were the moisture content, ether extractives (oils, fats, waxes, gums, and organic acids not further determined), alcoholic extractives (tannin, glucotannin, vanillin, and organic acids), nitrogen, starch, cellulose, and proteids. The paper contains a large number of analyses made at different seasons, but no general results have yet been reached, and it is difficult to see what may be expected. Part 3 of this volume is a small paper by ISTVÁNFFI[15] in which he describes a disease of the vine caused by *Phyllosticta Bizzozeriana* Massal. The disease is not of great importance, but has been mistaken for the black rot, one of the most dangerous vine diseases. In the part 4 ISTVÁNFFI[16] gives the results of his investigations on the gray rot, caused by *Botrytis cinerea*. The first part of this paper is taken up with the effects of various kinds of poisons and other treatments as cold, drying, etc., on the spores of the fungus. One of the most striking results is the unusually high resistance which the spores are said to have to copper. Spores were kept twenty-four hours in different strengths of Bordeaux mixture ranging from 1 to 10 per cent., to which was then added must containing 1 per cent. of tartaric acid, so that the resulting solutions contained the equivalent of 0.3 per cent. $CuSO_4$. Of the spores from the lowest strength mixture 38–40 per cent. germinated, of those in the highest 10–12 per cent. germinated. Spores sown on berries in 3 per cent. Bordeaux mixture germinated and penetrated the epidermis. Spores, kept one hour in a 2 per cent. solution of $CuSO_4$, which was then diluted with ten times its volume of must, germinated. Many other similar experiments are given. The second part of the paper deals with the development and life history of *Botrytis cinerea* and methods of control. Very little new is added to the life history of the fungus. For treatment, spraying with a 5 per cent. solution of calcium bisulfid is recommended.—H. HASSELBRING.

Endotrophic mycorhiza.—The long and important paper of GALLAUD[17] on this subject merits brief summary, as his conclusions are quite revolutionary. He has described for the first time the anatomical and cytological characters of

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[14] GASPAR, J., Analyses des sarments américains. **Ann.** Inst. Cent. Ampél. Roy. Hongrois **3**:57–166. *pls. 4–12.* 1905.

[15] ISTVÁNFFI, Gy. de, D'une maladie de la vigne causée par le *Phyllosticta Bizzozeriana*. *Idem*, 167–182. *pl. 13.* 1905.

[16] ISTVÁNFFI, Gy. de, Études microbiologiques et mycologiques sur le rot gris de la vigne. *Idem*, 183–360. *pls. 14–21.* 1905.

[17] GALLAUD, I., Études sur les mycorhizes endotrophes. Rev. Gén. Bot. 17: *pls. 4.* 1905.

a large number of endophytes, and his study enables him to distinguish four types: (1) type of *Arum maculatum*, hyphae intercellular after traversing the outer cells, their growth arrested by formation of simple terminal haustoria which penetrate the cortical cells; (2) type of *Paris quadrifolia*, hyphae intracellular, of indefinite growth, with complex lateral haustoria arising at definite points; (3) type of Hepaticae, hyphae intracellular, of indefinite growth, entering *via* rhizoids and bearing haustoria transformed into sporangioles; (4) type of Orchideae, hyphae intracellular, of indefinite growth, forming tight pellets which are sometimes permanent and sometimes undergo more or less complete digestion.

There is a remarkable uniformity in the constitution of the cell walls and in the cytological structure. Repeated attempts to isolate the fungi by direct extraction and by inoculation were unsuccessful. The first method failed, probably because the fungus already in was already too much altered by the digestive action of the host, and the second leads the author to distrust utterly the identifications of previous authors. The endophytes, he holds, are *saprophytes internes*, which by their highly differentiated haustoria borrow some non-living nutritive material from the cells in which they live. These cells react very rapidly on the fungus, killing its haustoria, digesting and absorbing them in part; then they resume their normal life, momentarily disturbed. It cannot be said that there is a harmonious symbiosis between the two plants, but rather a conflict between the invading, but little harmful, fungus and the cells which defend themselves by their digestive power.—C. R. B.

Sexual reproduction of Stigeoclonium.—PASCHER in an account of the sexual reproduction of *Stigeoclonium fasciculatum*,[18] touches briefly on the formation and behavior of the zoospores (macrospores), which in general agrees with that of other forms, but in a few cases the sporelings developed into filaments of a few cells only, which then formed in each cell a single four-ciliate zoospore (macrospore) that developed like other zoospores. The microspores are four-ciliate and long motile; after losing their motility they become spherical and either form resting-cells, or (rarely) conjugate and form zygotes. The development of the latter was not followed, but from hasty observation he concludes that their germination does not depart from that of the zoospores or the resting-cells. After an indefinite period the resting-cells germinate like the zoospores. Some, however, (akinetes or palmella stage), grow into a few-celled filament, each cell giving rise to four biciliate zoospores, resembling the microspores in size and activity, except that they will not conjugate but germinate at once like the zoospores.

Phylogenetically he claims for *Stigeoclonium fasciculatum* a position midway between Ulothrix and Draparnaldia, the three kinds of spores indicating that it is on the border-line of sexual reproduction. The same position was long ago claimed by DODEL-PORT for *Ulothrix zonata*. But such generalizations will bear

[18] PASCHER, A., Zur Kenntnis der geschlechtlichen Fortpflanzung bei Stigeoclonium. Flora **95**: 95–107. *figs.* 2. 1905.

revision, and investigations of the cytological phenomena involved are especially needed. PASCHER'S observations were microscopic to be sure, but he has apparently attempted no cytological observations at all.—R. THIESSEN.

Sigillarian stems.—Owing to the rarity of sigillarian stems showing structure the description of new specimens is of particular interest to paleobotanists. KIDSTON[19] has given a well-illustrated and adequate description of *Sigillaria elegans*, which differs from the historic *S. Menardi* in that the primary wood of the former is continuous instead of broken up into bundles. The protoxylem is external to the metaxylem, and both are composed of scalariform tracheids. The secondary wood is about equal in thickness to the primary, and shows medullary rays which are mostly one cell thick and one to nine cells high. The outer margin of the primary wood is crenate, and from the furrows arise the leaf traces, of which there are about twenty-eight in a cross section; these do not seem to possess any secondary wood. As is usual in sigillarian stems the pith, phloem, and inner cortex have perished, and the outer cortex contains a broad zone of periderm. *S. elegans*, with a continuous ring of primary xylem, *S. spinulosa*, with a mixture of continuous and discrete xylem, and *S. Menardi*, with separate bundles, form a good series, and judging from the scanty data available it seems that this series represents a sequence in time. The features of *S. elegans* support the view that the genus sprung from forms more like Lepidodendron.—M. A. CHRYSLER.

Mycoplasmic propagation of grain rust.—ERIKSSON has published another instalment of his studies on the demonstration of the propagation of grain rust by means of mycoplasm, this time dealing with *Puccinia graminis*.[20] Four means are recognized by which the uredo stage of the rust may possibly arise in spring time in winter wheat: (1) from spores of the barberry aecidium, which in turn arose from the resting teleutospores that had remained dormant over winter; (2) direct infection of the wheat plant from the resting teleutospores (homoecism); (3) uredo infection from mycelium remaining alive in the wheat plant over winter; and (4) from endogenous germs of disease (mycoplasm) which pass the winter in a resting condition in the live wheat plant. He marshals a large array of data, drawn from his own observations and experiments and from a wide range of literature, to show that the first method, although it exists, is by no means universal, that the second is highly probable, that the third never occurs in northern regions, if anywhere, and that the fourth is the most common method everywhere. Although the conclusions of the author will not be accepted by most investigators of this difficult problem, yet the array of data is interesting. Two clearly drawn

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[19] KIDSTON, ROBERT, On the internal structure of *Sigillaria elegans* of Brongniart's *Histoire des végétaux fossiles*. Trans. Royal Soc. Edinburgh **41**:533–550. *pls. 1–3*. 1905.

[20] ERIKSSON, JAKOB, Ueber das vegetative Leben der Getreiderostpilze IV: *Puccinia graminis* Pers. in der heranwachsenden Getreidepflanze. Kungl. Sv. Vet.-Akad. Handl. **39**[5]:1–41. *pls. 1, 2*. 1905.

colored plates are used to show the author's interpretation of the transformation of the resting mycoplasm into the mycelium condition of the rust.—J. C. ARTHUR.

Light relations at high altitudes.—WIESNER'S study of the *Lichtgenuss* of plants, already comprehensive for varying latitudes, has now been extended[21] to include high altitudes. During a period of thirty days from Aug. 16, photometric observations were made in the Yellowstone territory at eight altitudes ranging from 515 to 2210^{m} above sea level. The investigation shows that the behavior of plants with advancing latitude does not agree with that manifested under increasing altitude. The relative amount of available light appropriated by arctic plants increases inversely with the distance from the pole. This relation holds with increasing altitude only to a certain limit, above which a smaller and smaller share of available light is appropriated. The cypress habit of growth is evidently intended to protect from increased intensity of light, whether this accompanies low latitudes or high altitudes. This seems all the more probable because in such altitudes species having this habit do not show a defoliation from heat, which is manifested by other species that do not show it at lower levels.—RAYMOND H. POND.

Tomato rot.—VON OVEN[22] has recently described a disease of tomatoes caused by *Fusarium rubescens* Appel & Von Oven. This fungus causes a rotting of the tomato fruit, and evidently does not belong to the fungi in this group producing stem rot or wilt disease, although in cultures the pink and violet shades characteristic of the latter are also produced by this new species. As it is impossible to separate the species of Fusarium on morphological grounds, VON OVEN has attempted to distinguish this species at least from several disease-producing fusariums by their physiological characteristics. It is thus distinguished from *F. Solani*, *F. putrefaciens*, and *F. rhizogenum*. In cultures on sterilized potato small sclerotia were formed, which produced conidia after being exposed during December and January. The author concludes that this is a hibernating stage of the fungus, although he does not mention finding them in nature.—H. HASSELBRING.

Axillary scales of aquatic monocots.—As aquatic monocotyledons are by some held to be modern representatives of the more primitive angiosperms; as these forms may have been genetically related to some such type as Isoetes; and as he regards the ligule as an important phylogenetic organ, GIBSON[23] has made a study of the vestigial structures of the following families: Potamogetonaceae,

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[21] WIESNER, J., Untersuchungen über den Lichtgenuss der Pflanzen im Yellowstonegebiete und in anderen Gegenden Nordamerikas. Sitzungsber. Kaiserl. Akad. Wiss. Wien, Math.-Naturw. Klasse **114**[1]:(pp. 74.) *figs. 2.* 1905.

[22] OVEN, E. VON, Ueber eine Fusariumerkrankung der Tomaten. Landw. Jahrb. **34**:489–520. *pls. 5, 6. fig. 1.* 1905.

[23] GIBSON, R. J. HARVEY, The axillary scales of aquatic monocotyledons. Jour. Linn. Soc. Bot. **37**:228–237. *pls. 5, 6.* 1905.

Aponogetonaceae, Juncaginaceae, Alismaceae, Butomaceae, and Hydrocharidaceae. From an investigation of adult structure and manner of development, he has concluded that the axillary scales found at the bases of the leaves in the plants of these genera are homologous with the more specialized and solitary stipules of Selaginella and Isoetes. It will be recalled that GIBSON regards the ligule as a sort of specialized ramentum, protecting and keeping moist the young leaves and growing apex of Selaginella and Isoetes.—FLORENCE LYON.

Reserve food of trees.—NIKLEWSKI[24] confirms by macrochemical methods the observation of RUSSOW and of FISCHER, that in winter the fat-content of trees first increases and then diminishes. The process cannot be reversed by temperature changes. While a rise of temperature accelerates the formation of fat, no change affects its solution. The transformation of fat and of starch are not related. Low temperatures promote the formation of sugar from starch. Complex phenomena result from a rise of temperature. So great is the loss of reserves by the increased respiration, that it seems probable that bodies other than starch or fat share in the metabolism and give rise to carbohydrates.—C. R. B.

Conjugation of yeasts.—GUILLIERMOND[25] has extended his studies on the conjugation of yeasts to several additional forms of the Schizosaccharomyces and Zygosaccharomyces. The union of the cells is followed by the fusion of the two nuclei, after which the fusion nucleus divides and the two cells separate or spores are formed in the fusion cell. In some forms conjugation takes place with the germination of the spores. GUILLIERMOND regards this cell and nuclear fusion as a sexual act, but of course chiefly on physiological grounds. Since we do not know the history of the yeasts, it is a matter of speculation whether or not these conjugating cells are phylogenetically gametes.—B. M. DAVIS.

Amphispores in Uredineae.—ARTHUR has given an account of all species of rusts which have amphispores,[26] *i. e.*, as defined by CARLETON, one-celled spores which resemble the teleutospores of Uromyces in appearance, but have two or more germ-pores, and in germination behave like uredospores, their function seeming to be to tide the fungus over unfavorable conditions. This account includes one species of Uromyces and eight of Puccinia, one of which, *P. Garrettii*, is new. All the forms are American, for thus far no cases of the occurrence of amphispores have been reported from other parts of the world.—H. HASSELBRING.

Photosynthesis extra vitam.—BERNARD has again examined carefully the

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[24] NIKLEWSKI, B., Untersuchungen über die Umwandlung einiger stickstoffreier Reservestoffe während der Winterperiode der Bäume. Beihefte Bot. Centralbl. **19**[1]: 68–117. 1905.

[25] GUILLIERMOND, M. A., Recherches sur la germination des spores et la conjugaison chez les lévures. Rev. Gén. Bot. **17**: 337–376. *pls. 6–9. figs. 11.* 1905.

[26] ARTHUR, J. C., Amphispores of the grass and sedge rusts. Bull. Torr. Bot. Club **32**: 35–42. *figs. 9.* 1905.

question of photosynthesis *in vitro*, and again with negative results.[27] He repeated MACCHIATI'S experiments (following his directions *in litt.*), and tried also those of MOLISCH, which lent faint support to MACCHIATI'S conclusions. The gas disengaged seems due only to bacterial infection and when obtained at all does not conform in amount to that demanded by theory. This accumulation of negative results makes exceedingly doubtful the claims of FRIEDEL and MACCHIATI.—C. R. B.

Measuring transpiration.—CANNON describes[28] a method of studying the rate of transpiration upon plants in place, which he calls the polymeter method, because LAMBRECHT'S portable polymeter, a combined hygrometer and thermometer is used to ascertain the increase in humidity of the atmosphere around the experimental plant when enclosed in a bell jar. Certain defects in the method are noted, but the most important one, that it itself produces a variable decrease in transpiration, is not mentioned.—C. R. B.

Diastase.—KLEEMANN, finding the known methods of determining the course of diastase formation not sufficiently accurate, proposes a new, and, as he claims, more satisfactory one.[29] Using it he has determined that the amount of diastase formed depends, on the one hand, upon the water content of the barley, and on the other, upon how the water is supplied and taken up, and that the loss by respiration is greater the greater the water content.—C. R. B.

The sporophyte of mosses.—TRUE finds[30] that the nodding of the capsusel of Mnium, and probably of Funaria also, is due to geotropic stimulation, while the direction of illumination determines the plane of the curve in the seta, the apex of the capsule sometimes curving toward and sometimes away from the incident light. The calyptra affords important protection to the growing sporophyte from mechanical injury and desiccation.—C. R. B.

Chloroform a stimulant.—So Miss Latham[31] finds it in small quantities to Sterigmatocystis, especially at the time of germination, while larger quantities are inimical or fatal. Less acid formation and less sugar consumption under the stimulus indicate greater metabolic economy.—C. R. B.

Chromosome reduction.—A useful collective review of the recent literature on this subject is presented by KÖRNICKE in Bot. Zeit. **63**[2]: 289–307. 1905.—C. R. B.

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[27] BERNARD, C., Sur l'assimilation chlorophyllienne. Beihefte Bot. Centralbl. **19**[1]:59–67. 1905.

[28] CANNON, W. A., A new method of measuring the transpiration of plants in place. Bull. Torr. Bot. Club **32**: 515–529. 1905.

[29] KLEEMANN, A., Untersuchungen über Malzdiastase. Landw. Versuchsstat. **63**: 93–134. 1905.

[30] TRUE, R. H., Notes on the physiology of the sporophyte of Funaria and Mnium. Beihefte Bot. Centralbl. **19**[1]:34–44. 1905.

[31] LATHAM, M. E., Stimulation of Sterigmatocystis by chloroform. Bull. Torr. Bot. Club **32**: 337–357. 1905.

NEWS.

DR. ENRICO PANTANELLI has been appointed docent in botany at Rome.

EMILE BOUDIER, the eminent mycologist, has been elected director of the *Association internationale de géographie botanique* for the year 1906.

PROFESSOR Dr. A. RICHTER has been appointed director of the botanic garden of the University of Kolosvár, the post recently vacated by the death of Professor V. BORBÁS.

A PORTRAIT of Mr. FRANCIS DARWIN was lately presented to the botanical department of the University of Cambridge, where he was for many years an active investigator and instructor.

PROFESSOR HUGO DE VRIES will sail for New York about April 1, to deliver an address at the bicentennial anniversary exercises in honor of BENJAMIN FRANKLIN to be held in Philadelphia April 17–20, under the auspices of the American Philosophical Society. He expects to remain in this country two or three months.

DR. D. T. MACDOUGAL has resigned his position as assistant director of the New York Botanical Garden and has been appointed director of botanical research of the Carnegie Institute. Dr. B. E. LIVINGSTON has resigned his post as physiologist in the Bureau of Soils, U. S. Department of Agriculture, and Professor FRANCIS E. LLOYD his chair in the Teachers College of Columbia University, to accept appointments as investigators on the staff of the Desert Botanical Laboratory, with Drs. CANNON and SPALDING.

AFTER thirty years' service Sir W. THISELTON-DYER retired on December 15 from the directorship of the Royal Botanic Gardens, Kew, and was succeeded by Lieutentant-Colonel D. PRAIN, formerly director of the Botanical Survey of India, and superintendent of the Royal Botanic Gardens, Calcutta. Mr. DYER will remain at Kew till March 31 next, and till that date will continue to act as botanical adviser to the secretary of state for the colonies and as technical adviser in botany to the Board of Agriculture and Fisheries, as well as to take charge of India Office work.

FROM THE *Journal* of the New York Botanical Garden we learn that Mr. R. S. WILLIAMS has returned from two years' explorations of the Luzon, Jolo, and Mindinao, three of the Philippine Islands, bringing large and important collections of herbarium and museum material, estimated at ten to twelve thousand specimens, in spite of the loss of about three months' collections by fire.

DR. J. N. ROSE with an assistant, Mr. PAINTER, spent the summer in the arid districts of central and southern Mexico, collecting cacti, of which they

secured several hundred. Special arrangements are being made to study this family thoroughly, both in living and preserved material.

Some giant bamboos in the palm-house in the past season grew 65 feet (20^{m}) in ninety-five days, an average of about 21^{cm} per day.

FROM advance sheets of the seventeenth annual report of the Missouri Botanical Garden, we learn what extraordinary burdens the SHAW bequest has been carrying these sixteen years in the way of taxes, general and special, and real estate and street improvements. This has unhappily delayed the design of Director TRELEASE for development of the Garden as a research center, making impossible the prompt execution of the plan to maintain a staff of specialists and furnish them facilities for work. If the city and state were as just as Mr. SHAW was generous they would relieve the Garden of taxes at least, since it exists solely for the public good. Notwithstanding these unexampled inroads upon its income the institution has not stood still; the garden has not only been maintained but greatly improved; a fine library and herbarium has been accumulated, and notable researches have been published annually. The grounds now embrace 65 acres, the plant houses cover 30,000 square feet, the cultivated plants number 16,000 species, noteworthy groups being the cacti (678 sp.), bromeliads (204 sp.), and orchids (942 sp.). The library is now undoubtedly the best botanical library in the United States, and the herbarium contains over half a million specimens. We congratulate the Director and Trustees on the wise administration of their trust in the face of serious difficulties and discouragements.

THE American Mycological Society held its third annual meeting in connection with the American Association for the Advancement of Science at New Orleans, January 1, 1906. In the absence of the president, CHARLES H. PECK, the vice-president, F. S. EARLE, presided The new constitution recommended by the joint committee of the Botanical Society of America, the Society for Plant Morphology and Physiology, and the American Mycological Society, as a basis for the union of the three societies, was adopted and the present officers continued as a committee with power to cooperate in the completion of the details of reorganization. The following papers were read: J. C. ARTHUR, Some reasons for desiring a better classification of the Uredinales; S. M. TRACY, Uredineae of the Gulf States; W. G. FARLOW, Some peculiar fungi new to America; F. S. EARLE, North American gill fungi; BRUCE FINK (by title), Lichens and recent conceptions of species; E. M. FREEMAN, The affinities of the fungus of *Lolium temulentum;* C. L. SHEAR, *Peridermium cerebrum* Peck, and *Cronartium Quercuum* (Berkeley); C. L. SHEAR, Romularia: An illustration of the present practice in mycological nomenclature; P H. ROLFS, Notes on cultures of Collelotrichum and Gloeosporium; P. SPAULDING, The occurrence of *Fusoma parasiticum* Tubeuf in this country; P. H. ROLFS, Notes on *Pachyma cocos;* P. H. ROLFS, *Penicillium glaucum* on pineapple fruit.—C. L. SHEAR.

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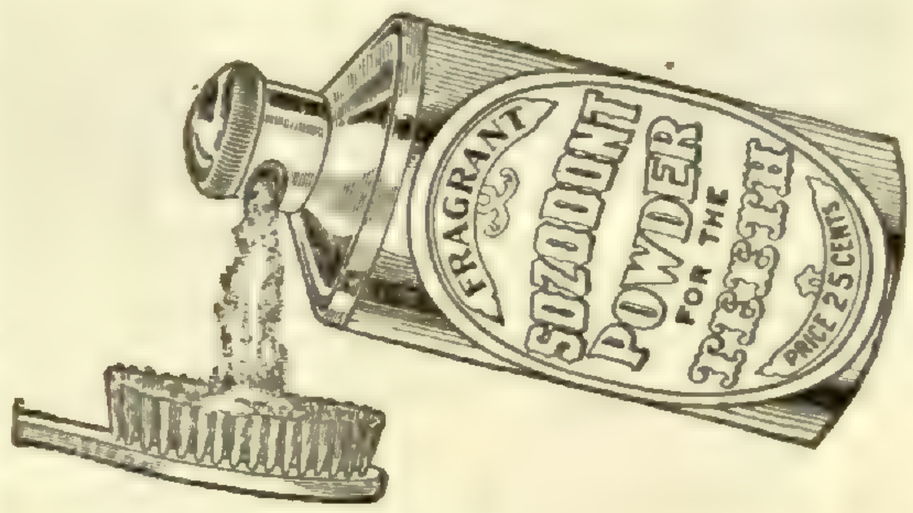

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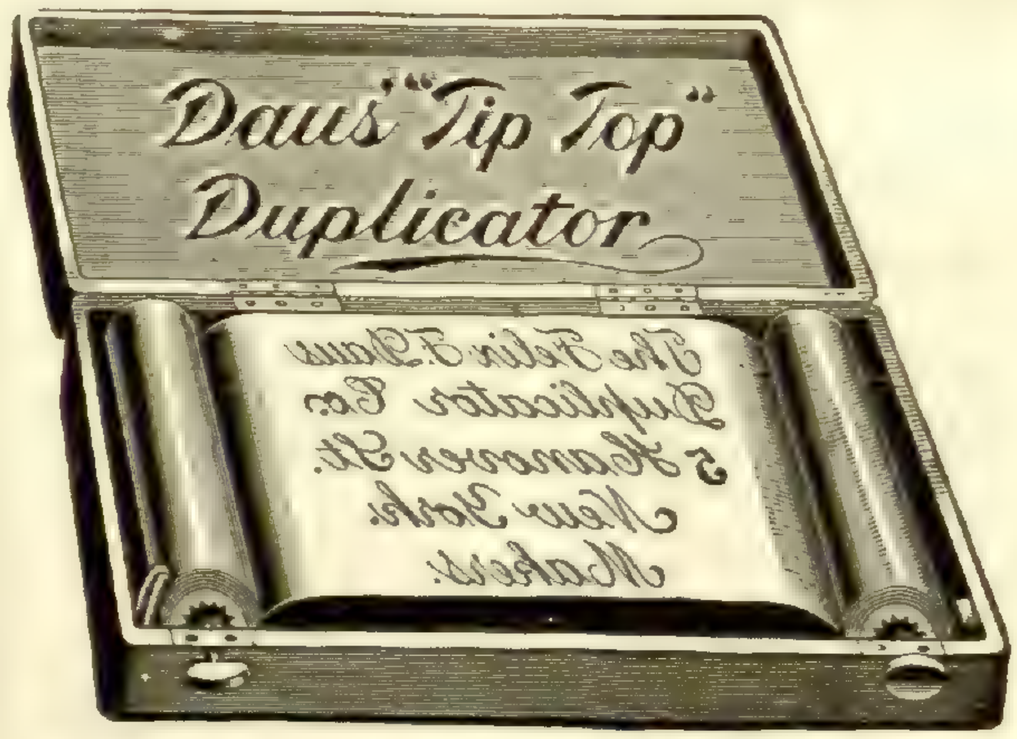

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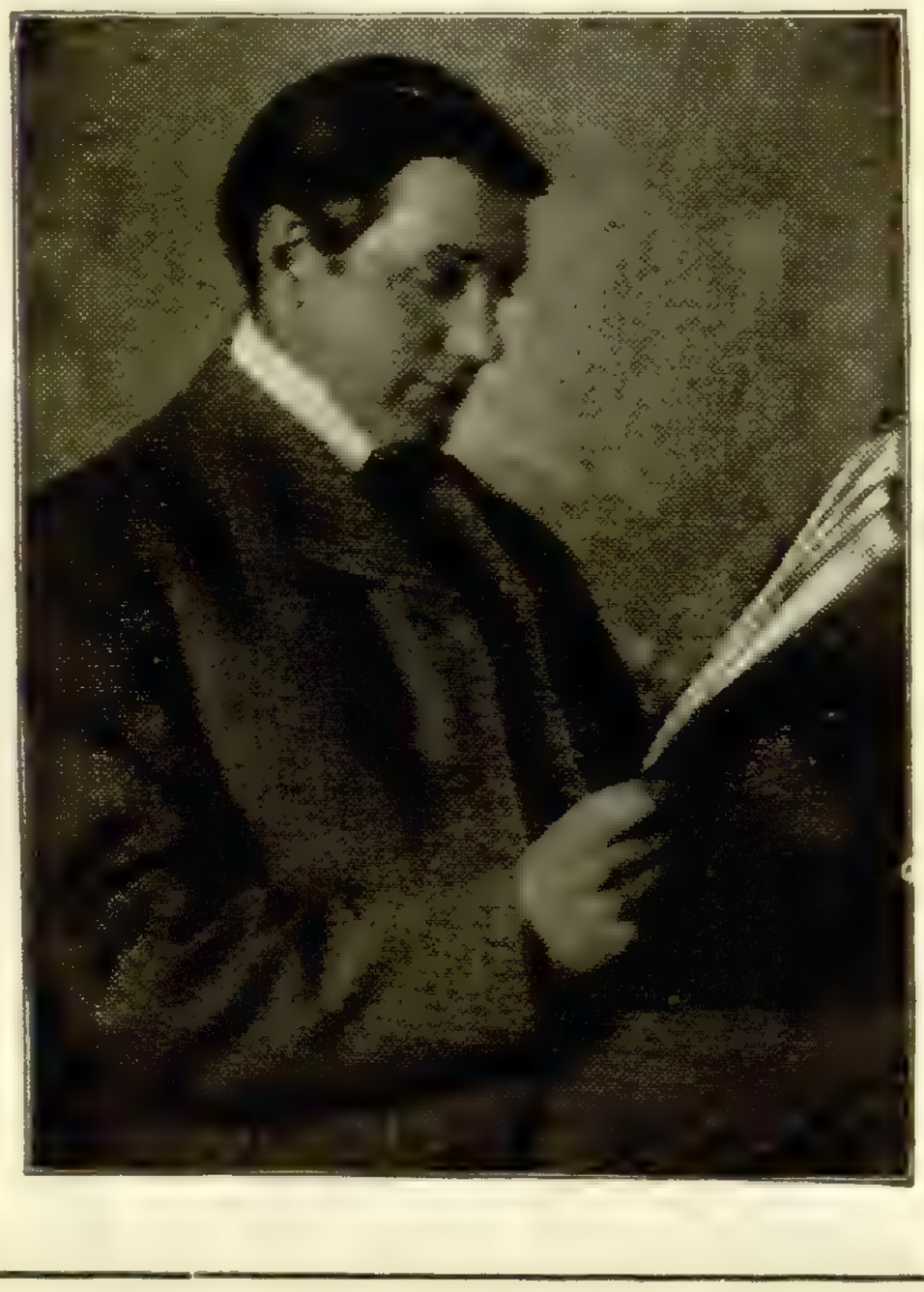

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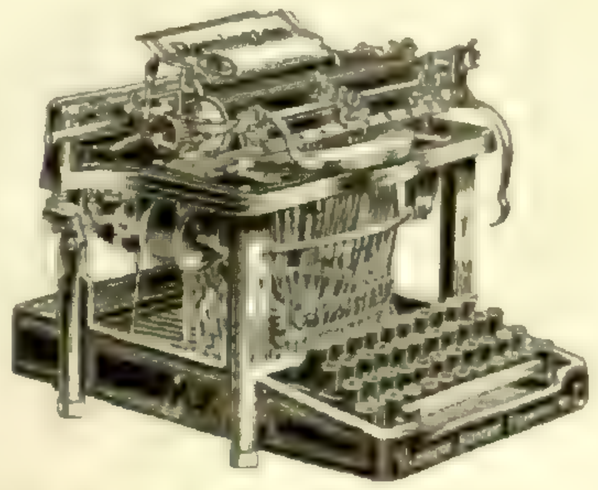

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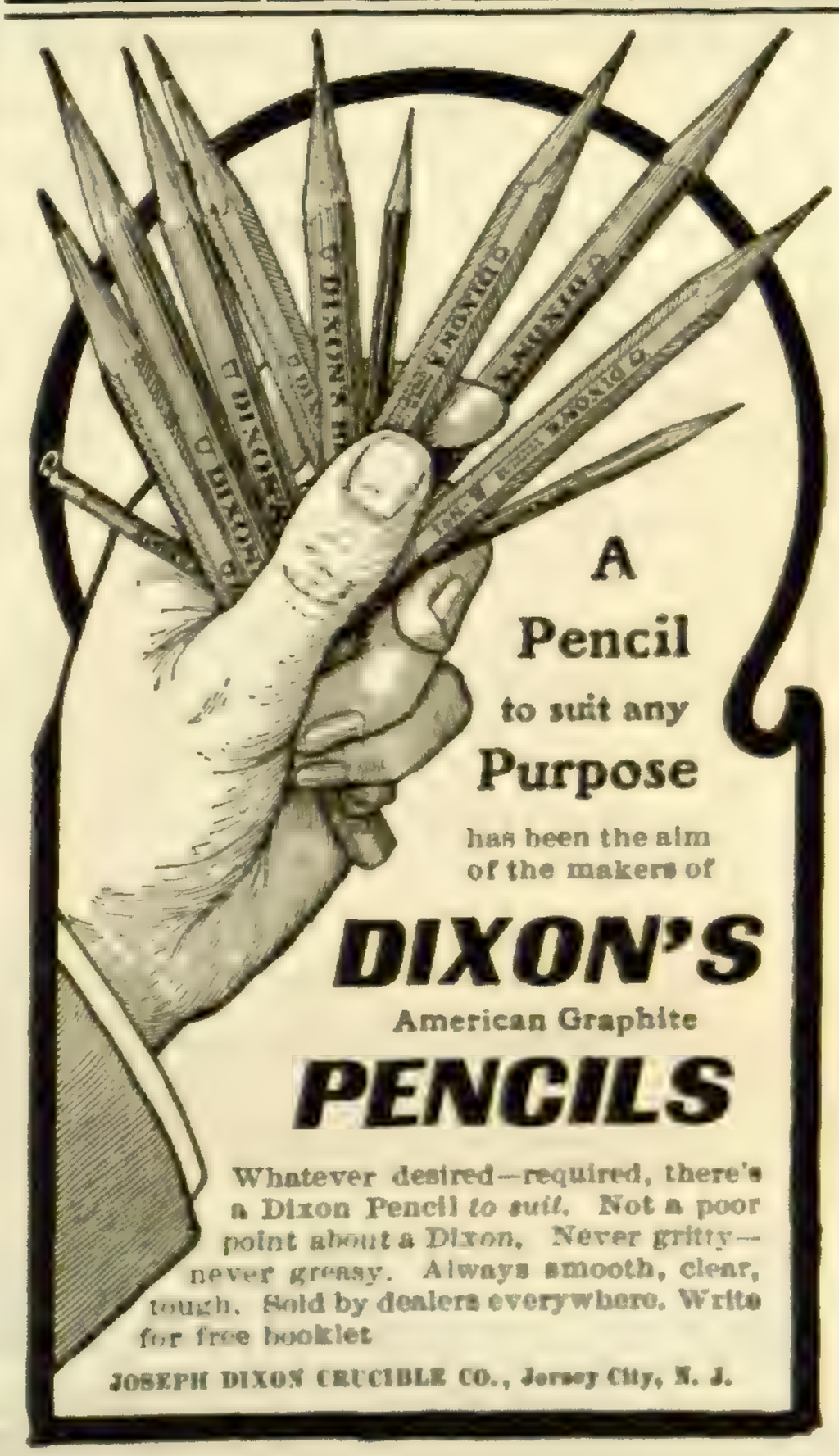

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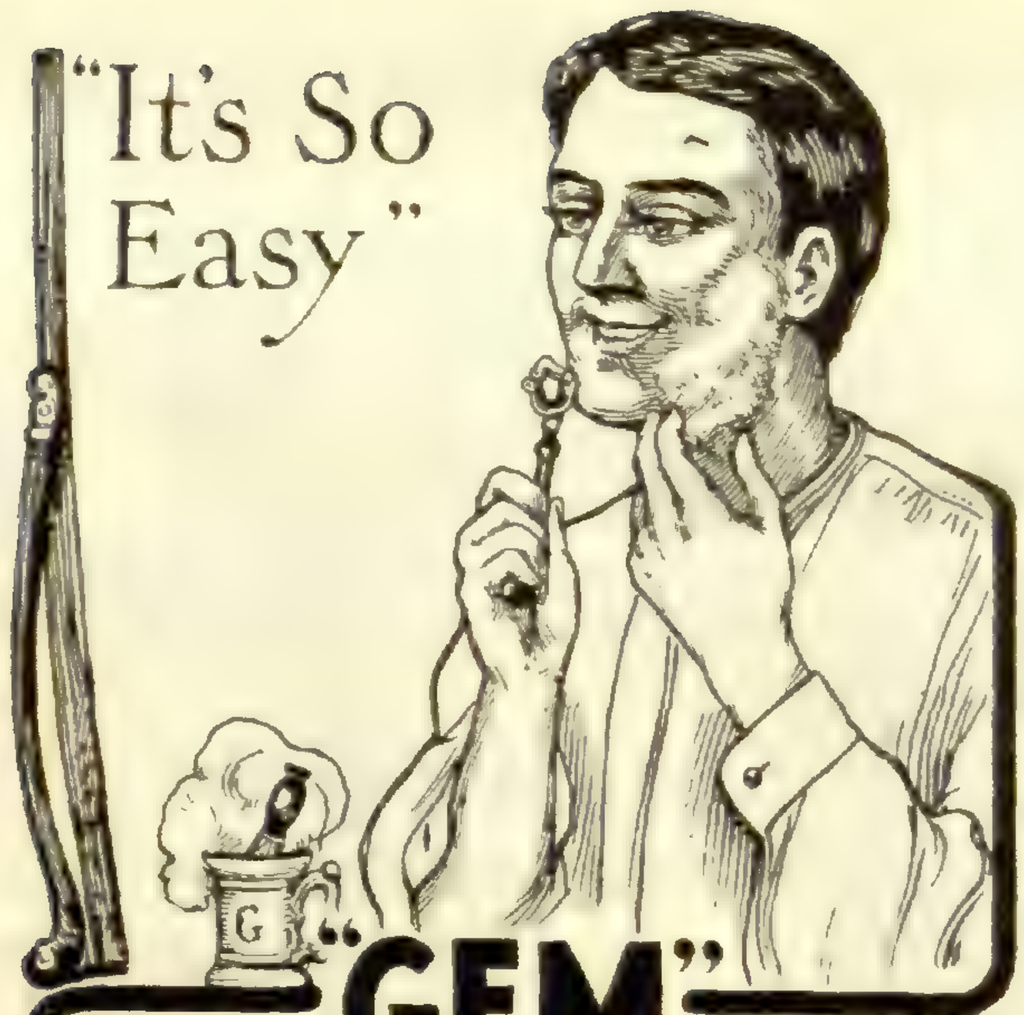

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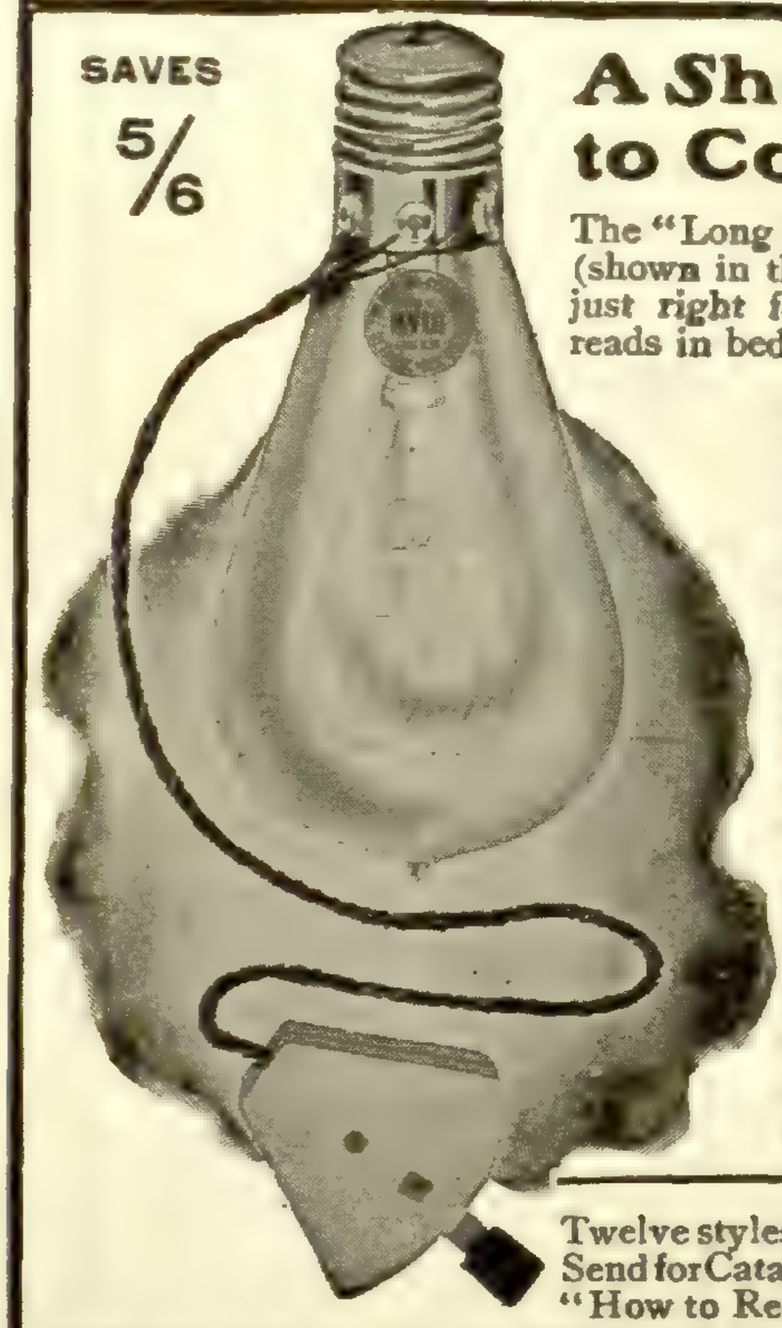

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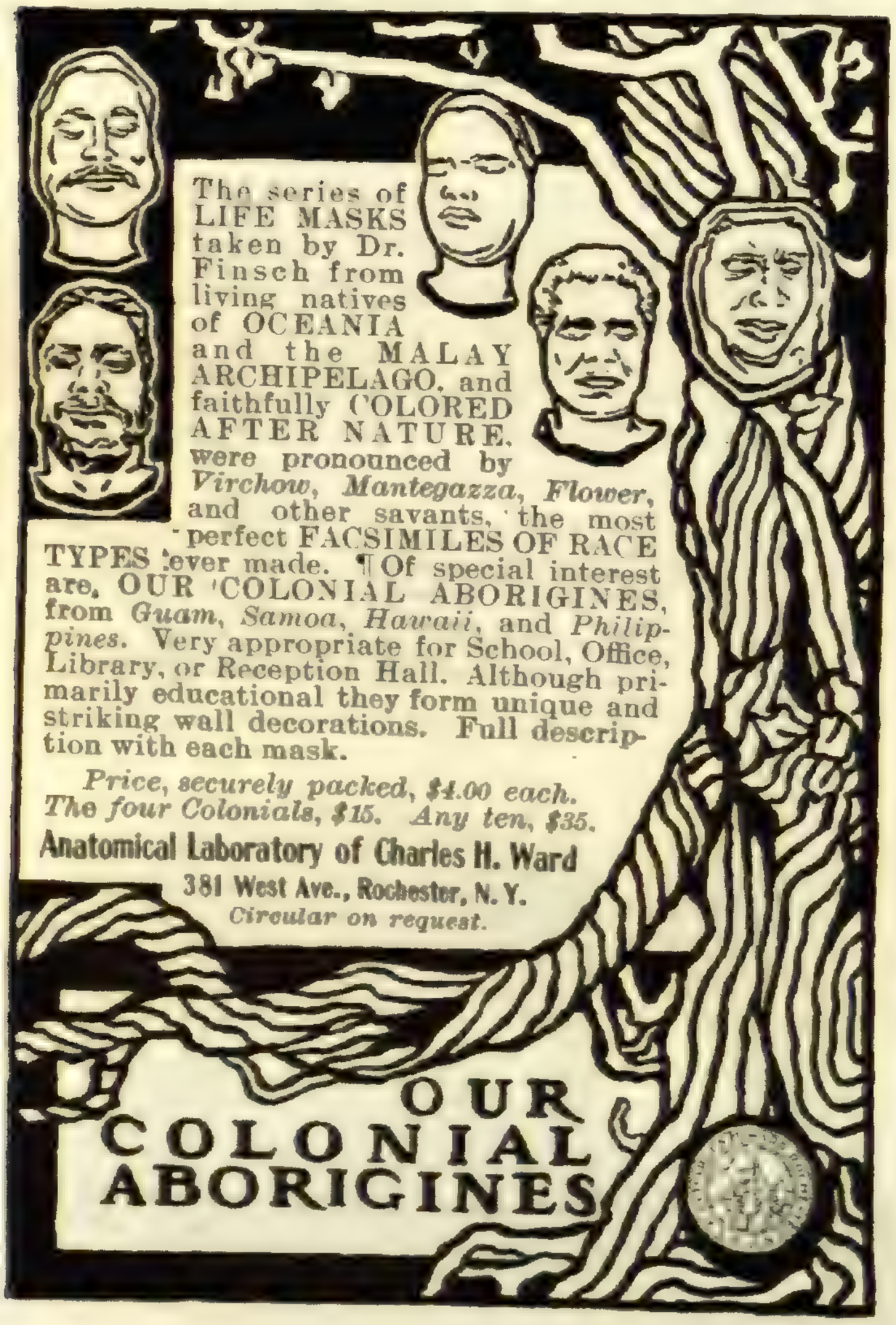

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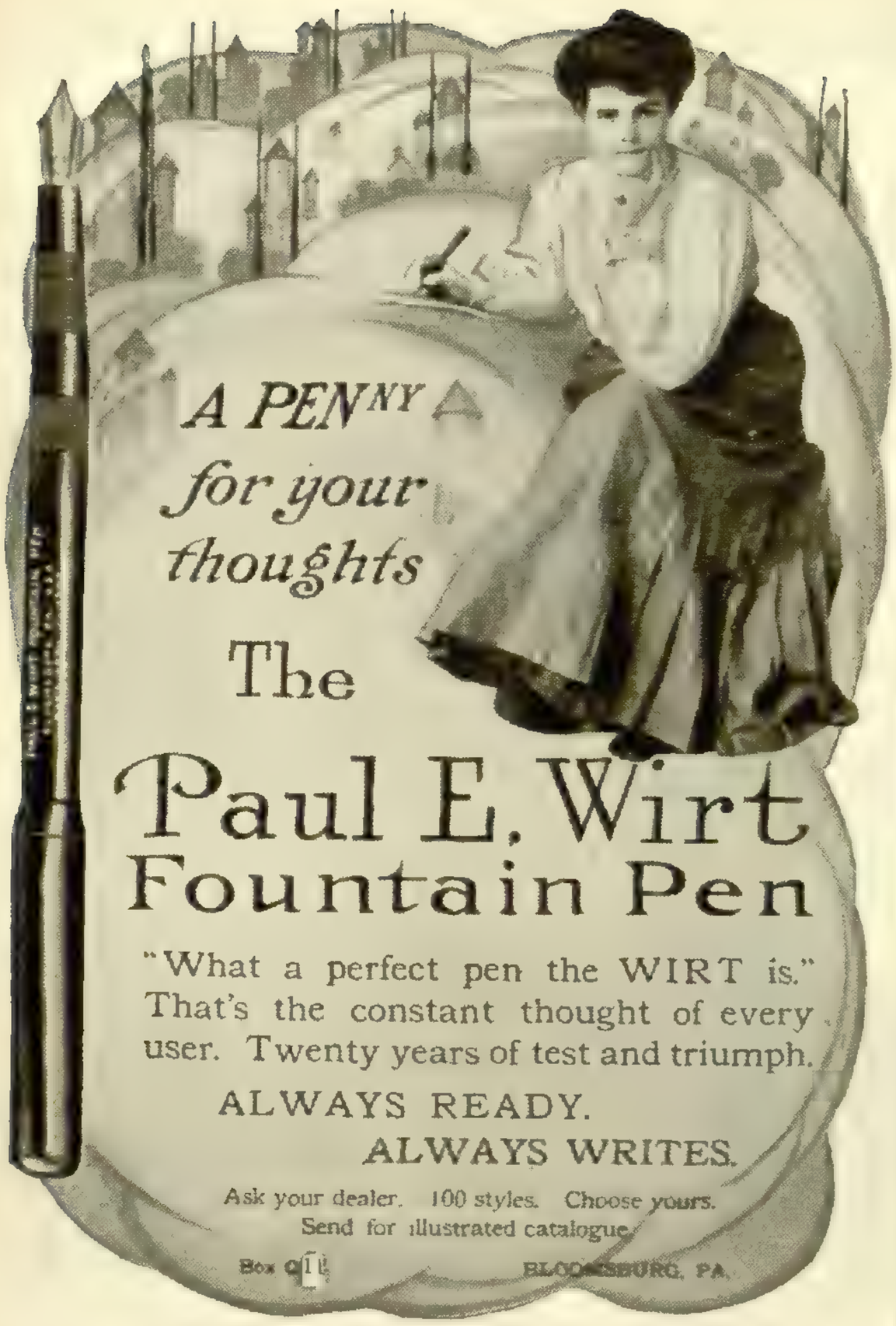

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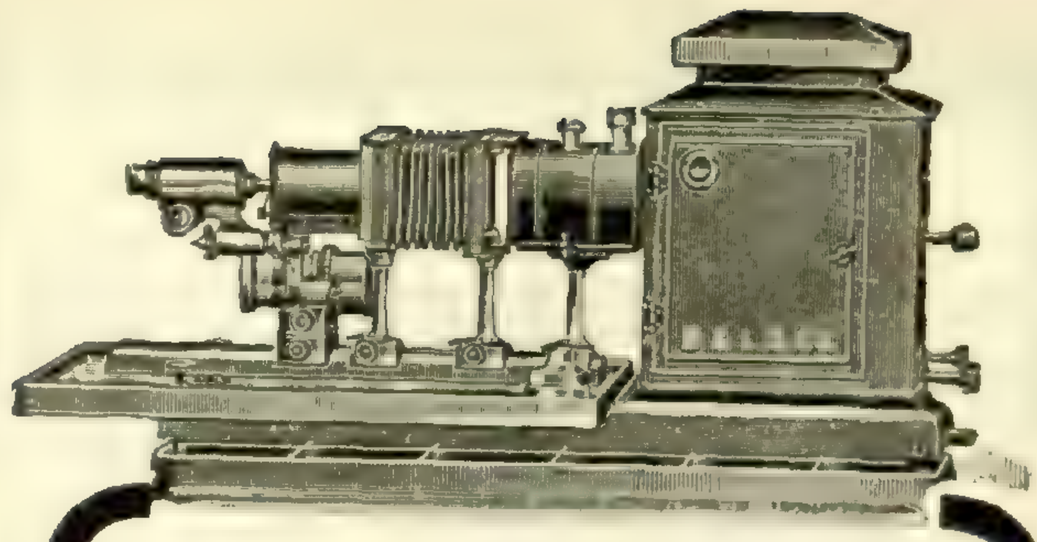

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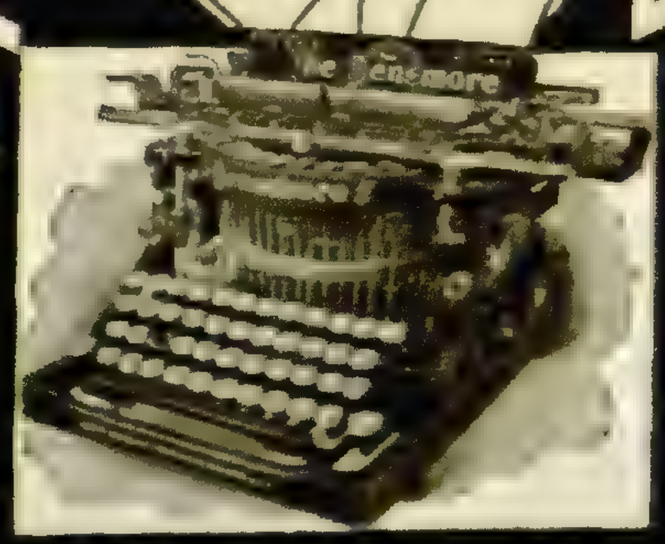

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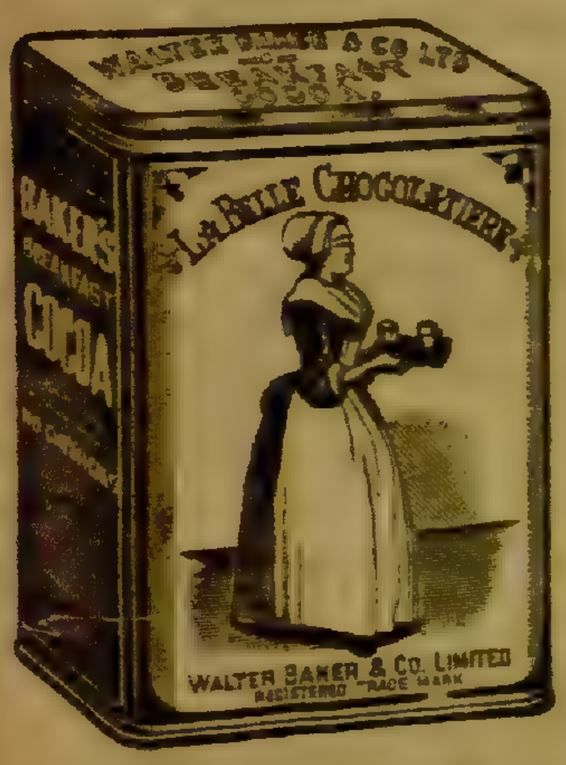

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March, 1906

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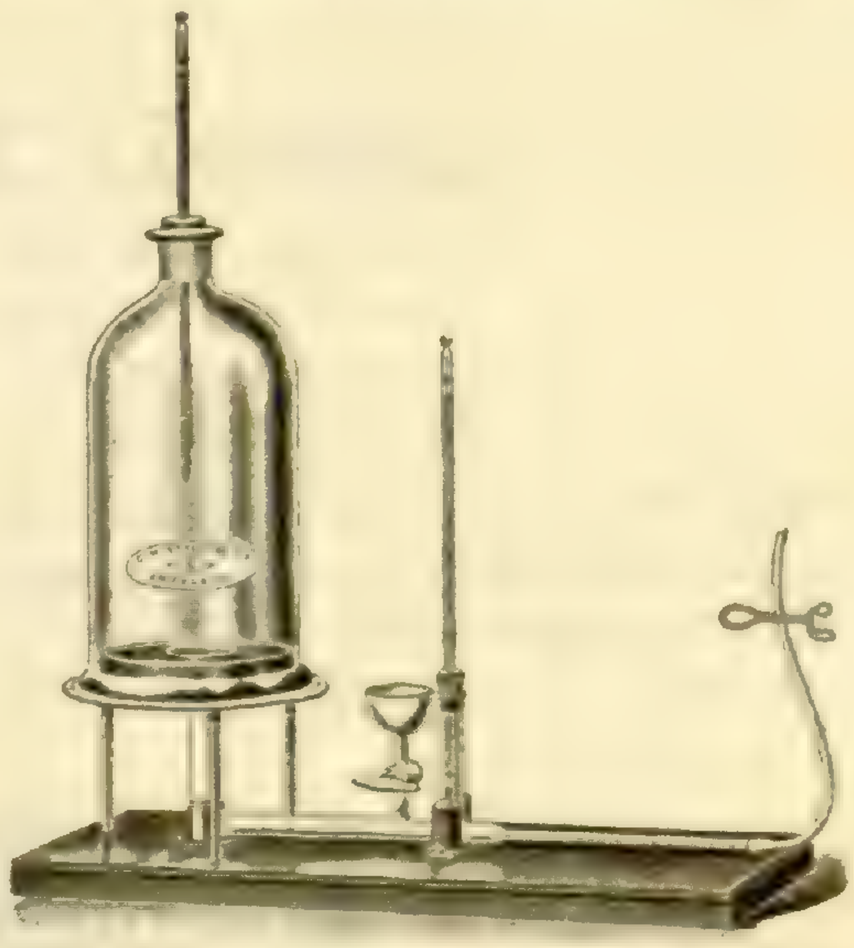

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VOLUME XLI NUMBER 3

BOTANICAL GAZETTE

MARCH, 1906

A MORPHOLOGICAL STUDY OF SARGASSUM FILIPENDULA.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXXII.

ETOILE B. SIMONS.

(WITH PLATES X AND XI)

THE family Fucaceae is less understood than its position and prominence in the Phaeophyceae warrant. Many important types have scarcely been considered at all, and, moreover, aside from the comparatively recent cytological studies in the family, few investigations have been conducted with modern methods of technique. The problems of morphology and cytology in the Fucaceae center chiefly around the sexual organs; the peculiar sunken structures in which they are borne, termed conceptacles; the likewise sunken but sterile structures called cryptostomata; and the sporelings.

The present investigation of these structures in *Sargassum filipendula* Ag., a member of perhaps the most highly differentiated genus in the Fucaceae, was undertaken with the hope of filling some of the obvious gaps in our knowledge of this family. It was conducted in the University of Chicago and at the Marine Biological Laboratory, Woods Hole, Massachusetts, under the direction of Professor BRADLEY MOORE DAVIS, who suggested the research to me. It gives me pleasure to express here, both to him and to Professor JOHN MERLE COULTER, my appreciation of valuable suggestions and assistance given me in this work. My acknowledgments are also due the Carnegie Institution for the use of a table at the Marine Biological Laboratory during the summer of 1904.

References to anatomical and morphological work which concern

this subject will be given under the topics to which they belong. The early history of the genus with its taxonomic bearing is omitted, as having no place here, but the once credited distribution of Sargassum which was convincingly disproved by KUNTZE ('81) is a matter of history which deserves at least brief mention.

KUNTZE relates that LINNAEUS believed that a vast area of sea was densely covered by Sargassum in active vegetative condition; HUMBOLDT reported that the region surpassed Germany in extent six or seven times; MAURY stated that it equaled the Mississippi valley; and HOECKEL estimated its area to be forty thousand square miles. That these views were generally accepted is well known. They led to instruction regarding a "Sargasso Sea," whose supposed limits were outlined upon maps of the world. KUNTZE, by comparing his own observations and those of other travelers over routes which crossed in different places the outlined area, was able clearly to disprove the existence of such a "sea." Sometimes a voyage was made through the mapped region and little or no Sargassum was seen, and again it appeared somewhat abundantly, but without definite limits or fixed location. Storms which sweep tropical shores, near which attached Sargassum grows abundantly, were found to be in great part accountable for the appearance of the larger quantities of floating Sargassum. KUNTZE obtained no evidence to substantiate the view that floating Sargassum vegetates. It had been believed that floating forms of Sargassum consisted of *S. bacciferum* only, but KUNTZE found several species floating, and observed that the specimens in herbaria which had been collected in mid-ocean and labeled *Sargassum bacciferum* according to general belief, could be referred to various species. He therefore concludes that there is no characteristic floating species. The appearance in mid-ocean of floating masses now and then does not seem strange when the authentic distribution and abundance of attached Sargassum are recalled. According to KJELLMAN ('93) this genus, which includes one hundred fifty species, over half the number belonging to the entire family, is found attached along the coast of all warm seas, reaches north to Cape Cod in the Atlantic, to Japan in the Pacific, and in the south into Australian waters, where it is the most abundant. With the extent of this distribution in mind the presence of floating masses, especially after storms, is to be expected.

MATERIAL AND METHODS.

Material for this study was collected near the shores of Woods Hole, late in July and during August. Plants both in vegetative and in reproductive conditions were abundant. The weak solution of chromacetic acid of Flemming (1 per cent. chromic acid 25^{cc}, 1 per cent. acetic acid 10^{cc}, water 65^{cc}) proved a satisfactory killing and fixing reagent. Microtome sections were cut from paraffin 5 μ in thickness and stained either by iron-alum-haematoxylin after the method of Heidenhain or by safranin and gentian violet. The mucilage on the surface of the plant and in young conceptacles and cryptostomata takes the anilin dyes readily, but is not especially troublesome.

GENERAL MORPHOLOGY AND HISTOLOGY.

The habit of *Sargassum filipendula* is so like that of other species which have been described that it needs but slight attention. This species grows attached to rocks below low water mark, and therefore, unlike Fucus and Ascophyllum, is never exposed to the air. Vegetative plants and reproductive plants bearing all stages of conceptacles are plentiful in summer. Sporelings are abundant also and easily collected, for the discharged eggs and their products, the sporelings, remain attached for some time by mucilage to the surface of reproductive branches near the parent conceptacles.

The stem arises from a small disk-shaped holdfast and passes into long cylindrical branches which bear spirally arranged leaves, berry-like floats, which seem to be modified portions of leaves, as generally stated, and short reproductive branches. This form may attain a height of 60^{cm}, but is commonly shorter. Cryptostomata develop upon stems, leaves, and occasionally also upon reproductive branches in Sargassum, which differs in this respect from Fucus, whose receptacles, according to BOWER, contain no cryptostomata.

KJELLMAN ('93) states that the conceptacles of Sargassum are hermaphrodite. In *Sargassum filipendula* both mature bisexual and unisexual conceptacles are formed. Some conceptacles contain only spermatocysts (antheridia); some, more rarely, contain many spermatocysts and but one or two oocysts (oogonia); and others bear only oocysts. The appearance of a conceptacle devoted to the formation of oocysts differs decidedly from such a structure in Fucus. In

Sargassum the oocyst has no stalk cell. It is an embedded organ, being almost surrounded by wall cells of the conceptacle. As both the size and contents of a conceptacle are dependent upon the activity of wall cells (as described later), this conceptacle in Sargassum is smaller and has fewer sexual organs and paraphyses than the corresponding conceptacle in Fucus. The unisexual tendency in the conceptacle of Sargassum may be due in part to the unproductiveness of the many wall cells which abut upon the embedded oocyst.

The anatomy of the thallus of Sargassum has been studied in four species. In 1876, REINKE reported its development in *Sargassum Boryanum* from a three-sided apical cell situated at the bottom of a pit in the apex of the stem. He stated that the holdfast is composed of rhizoids and that a few intercellular filaments occur in the old parts of the thallus. OLTMANNS ('89) in an anatomical investigation of *Sargassum linifolium* and *S. varians*, likewise described a three-sided apical cell, and in addition gave an account of the origin both of the apical cell of a leaf and of a branch. He believes that the branching in Sargassum holds no relation to dichotomy. He figures an enlarged epidermal cell near the apical cell of the stem, and states that it becomes a three-sided apical cell. This young cell develops an outgrowth in which a second apical cell is soon differentiated, between the first and the stem. The first formed apical cell develops a leaf and the last a branch. OLTMANNS agrees with KUNTZE ('81) that there are all gradations between leaves and floats, and that floats are modified portions of leaves.

In 1892, HANSTEEN published the results of an anatomical and physiological investigation of *Sargassum bacciferum*. He also reported a three-sided apical cell, but did not trace its origin in any structure. He described three kinds of tissues, naming them the assimilating system, the storage system, and the conducting system. The assimilating system, according to HANSTEEN, includes only the outer layer of cells, or epidermis. Its cells are twice as long as broad, have undulating walls, like the epidermal cells in higher plants, and contain "phaeoplasts." The cells of this system add to their own number by radial, and to the cells below by tangential, divisions. The storage system occupies a zone several cells wide between the assimilating system and the innermost tissue which constitutes the conducting

system. Most of the cells in the storage system are large. HANSTEEN found them empty in alcoholic material of Sargassum, but he did not doubt their function to be that of storage, because he had found much reserve material in similar cells of living Fucus. The conducting system consists of an axial cylinder of long cells with small diameter and oblique end walls. These cells are believed by HANSTEEN and others to function as sieve tubes. The cells of the three systems communicate by pores.

HANSTEEN observed in the storage cells of *Fucus serratus* and several other types, spherical grains of different sizes, which he named fucosan. He believes that the same structures have been variously considered as fat, proteid, and starch by other observers. The grains do not stain blue with iodin, and are soluble in water. HANSTEEN, who made a chemical analysis to determine their composition, considers them as a carbohydrate with the formula $(C_6H_{10}O_5)_n$. CRATO ('**92**) described in *Chaetopteris plumosa* spherical or elliptical bladder-like structures which he named physodes. He reported ('**93**) that they contain phloroglucin as a constant ingredient, function in directing the chemical exchange and transportation of food material within the cell, have motion, and are independent cell organs like the nucleus and chromatophore. CRATO stated further that HANSTEEN had confused various cell contents, and that fucosan grains and physodes are the same. KOCH ('**96**) denied the presence of phloroglucin in these bodies. In a later paper HANSTEEN (:**00**) again discusses fucosan grains. He maintains that CRATO's physodes are fucosan grains, and that they are not independent cell organs but products of the phaeoplast. HANSTEEN has made no further chemical analyses to determine the nature of the bodies, but holds that they surely represent a product of photosynthesis. HANSEN ('**95**) after an investigation of several forms (*Dictyota dichotoma*, *Taonia alomaria*, *Halyseris polypodioides*, Asperococcus, Hydroclathrus, and Cystoseira), states that the Phaeophyceae contain oil and no starch, and OLTMANNS (:**04**) expresses the same view. It is seen therefore, that the character of the reserve material in the cells of the Phaeophyceae is still somewhat problematical.

Every stem and leaf structure in *Sargassum filipendula*, as in other species studied, develops through the activities of a three-sided apical

cell. The tissue systems described by HANSTEEN are present and each seems to have the function ascribed to it, although without rigidity. Each system, too, has its origin in the group of segments surrounding the apical cell and can be traced very near it. The cells of every system are meristematic in the apical region, but the epidermal cells are apparently the only ones which retain this activity. The cells of any one of the three systems correspond well in general appearance with the similarly placed cells described by HANSTEEN, but an interesting modification was observed in the cells of the conducting system. All are long and of small diameter, but in respect to thickness of walls the tissue is differentiated into two regions. The inner cells have thin walls, while the outer ones have thick walls. The thick-walled cells may be both supporting and conducting in function. The conducting system of a leaf blade consists only of thin-walled tissue. No intercellular filaments, as reported by REINKE, have been found. Sometimes, however, a filamentous alga creeps into the mucilaginous walls of cells near the surface of a leaf or old stem, and gives the appearance of intercellular filaments. As the little alga contains true starch, its cells when stained with iodin present a sharp contrast to the unstained cells of Sargassum. HANSTEEN ('92) figures pores in thin areas consisting of the middle lamella in *Sargassum bacciferum*, and REINKE ('76) represents similar areas but without pores in cell walls of *Fucus vesiculosus*. Such thin areas are common between cells in the tissues of *Sargassum filipendula*, but pores, though probably present, are rarely seen.

The character of the reserve material in Sargassum proved of great interest. Sections from plants which have been preserved in formalin contain much more stored material than tissues which have been kept in alcohol. Preparations, however, which have passed through alcohol, xylol, paraffin, the heat of the bath, etc., still contain within the cells of the epidermis and outer cortex, many bodies which in all probability represent reserve food material. These bodies, which stain readily, vary in size and structure, but are evidently related, for transitional stages can be found between the most extreme forms. Judging by the appearance of the structures, some are intact and others modified. Those which seem intact are spherical, with a diameter which equals or exceeds the length of a chromatophore. Each con-

sists of a more or less homogeneous ground substance and one or more refractive areas which are somewhat centrally placed. The modified structures vary from spheres, whose ground substance has been changed only at the periphery, to swollen masses which have an entirely modified ground substance with an irregular outline. Both the intact and modified bodies may occur within the same cell; but the former and the least modified are more common in epidermal cells, whereas the most modified are in cortical cells. The occurrence of such bodies within epidermal cells where photosynthesis is the most active, suggests that they represent a manufactured food . The varied modifications in the structures indicate the solvent action of the killing fluid, or an intercellular enzyme. As the inner cells contain bodies presenting greater modifications than the epidermal cells, the agent producing the change is apparently applied from within the tissue. If then within, it is probably an enzyme, for a solvent used in the process of killing would attack the contents of epidermal cells, doubtless before any others. The intact bodies may represent a newly formed product, perhaps a carbohydrate, and the modified structures, the product in process of digestion. The bodies do not stand with iodin in any condition. If they are carbohydrate they probably differ as much or more from the starch of higher plants as does inulin. The presence of many small spheres in formalin material and their absence from tissues preserved in alcohol indicates that oil globules are present in the cell, in addition to the structures described above. Future investigations on living material will probably disclose the presence of both oil and a carbohydrate in the Phaeophyceae.

THE ORIGIN AND DEVELOPMENT OF THE CONCEPTACLE.

The conceptacle in the Fucaceae had been but little studied when BOWER ('80) gave an account of its development in four genera and six species (*Fucus serratus*, *F. platycarpus*, *F. vesiculosus*, *Ozonthallia nodosa*, *Halidrys siliquosa*, and *Himanthalia lorea*). According to him the development of the conceptacle in every species conforms to one scheme with minor variations.

The "initial cell" of the conceptacle, as stated by BOWER, is the terminal cell of a linear series which is produced by a modification of the regular divisions in the segments of the apical cell of a receptacle.

This initial cell, strangely, contributes nothing essential to the conceptacle. It either degenerates directly without having divided at all, or it produces a short filament whose terminal portion degenerates. A cortical cell below the initial is termed by BOWER a "basal cell." This cell and others which adjoin the initial cell laterally, divide and form the walls of the conceptacle from which the sexual organs and paraphyses arise. The initial cell, therefore, according to BOWER, takes no part in the development of the conceptacle, whereas the cells adjacent to the initial produce all that is important, the walls and their products. The prominent features of this scheme for the development of the conceptacle are, it is seen, degeneration of an unimportant initial cell or a part of its filamentous product, and the activity of cells adjacent to the initial in producing the entire conceptacle.

Nearly all contributions in this field since 1880 have been in the main confirmatory of the work of BOWER. VALIANTE ('83) states that the development of the conceptacle in Cystoseira is due to the growth of neighboring tissue, about one or two cells. OLTMANNS ('89) describes the walls of the conceptacle of *Halidrys siliquosa*, *Himanthalia lorea*, and *Ascophyllum nodosum*, as also formed by neighboring cells, with the one exception that in Ascophyllum the initial cell develops a mass of tissue in the base of the conceptacle. This tissue, he reports, shares with the rest of the inner surface formed from neighboring tissue, in developing the sexual organs. As no degeneration of tissue was observed in Ascophyllum, and as its initial cell does contribute some important tissue the development of the conceptacle, this genus presents an exception to a part of the scheme which BOWER reports. Although Sphlachnidium should no longer be included in the Fucaceae, as shown by the Misses MITCHELL and WHITTING ('92), it is of interest to note that these investigators report its conceptacle as developing by the radial division of cells adjacent to a persistent but inconsequential element, which they believe to be homologous with the initial cell of BOWER. GRUBER ('96) states that the conceptacle of *Seirococcus axillaria* is more like that of Halidrys than Ascophyllum, which means, again, that it has an initial cell which contributes nothing of consequence to the conceptacle, whose walls are formed by cells which are adjacent to the initial.

HOLTZ (:03) reports that in the development of the conceptacle of

Pelvetia fastigiata several epidermal cells cut off basal segments which divide transversely until six or more tiers are formed. Over these tiers, one or more epidermal cells break down and a cavity results, which is gradually enlarged by further disintegration of epidermal and meristematic cells. After a time this process ceases, and a "healthy surface" is formed from the deeper meristematic cells. This surface, which comprises the walls of the conceptacle with the exception of the upper part that is formed by "cortical rows" of cells, produces sexual organs and paraphyses. The prominent features which distinguish the conceptacle of Pelvetia from others, as thus described, are the presence of several epidermal or initial cells, the more extended disintegration of tissue, and a difference in the behavior of the basal cells.

The development of the conceptacle in *Sargassum filipendula* is at variance with all the prominent characteristics in the development of the conceptacle as described by BOWER. The initial cell of Sargassum does not break down. It is an active cell which produces the entire conceptacle. As the whole conceptacle is the product of this one cell, adjacent cortical tissue takes no part whatever in the development of the structure. The first indication of the conceptacle is a clearly differentiated epidermal cell which lies near the apical cell of a reproductive branch (*fig. 1*) and constitutes the initial cell of the conceptacle. The upper portion is surrounded laterally by epidermal tissue, whereas its central and basal regions are bounded by cortical. The initial is much larger than any of the cells with which it is in contact and differs much from them in shape. Though it may vary somewhat in length it is always flask-shaped. Its oval bowl, sometimes slightly narrowed at the base, tapers above into an elongated neck whose outer end is flush with the surface. The initial cell is circular in cross section at its apex (*fig. 1a*) and elliptical at its base (*fig. 1b*).

The initial cell never breaks down. On the contrary the development of the conceptacle is initiated by its activity. Its large nucleus divides. Then a curved wall is formed with concave surface above, separating two very unlike cells (*fig. 2*), which form the two-celled stage of the conceptacle. The upper cell, which may be designated the tongue cell, is a long somewhat cylindrical structure; whereas the lower is somewhat conical or wedge-shaped. The initial cell and the two-celled stage of the conceptacle have similar outlines both in longi-

tudinal (*figs.* 1 and 2) and in transverse sections (*figs.* 1*a*, *m*, *b*, and 2*a*, *m*, *b*). That the lower portion of the tongue cell is surrounded by the upper part of the cell below it is well shown in both longitudinal and transverse sections of the two-celled stage of the conceptacle.

The lower cell of this two-celled structure divides longitudinally into two similar daughter elements, thus producing the three-celled stage of the conceptacle (*fig.* 3). The longitudinal wall reaches to the lower portion of the tongue cell, whose basal portion is surrounded now by two cells instead of by one. The relative position of the three cells is made clearer by an examination of their transverse sections. A cross section near the base of the three-celled structure shows two similar cells (*fig.* 3*b*). A cross section about midway between the apex and base shows three cells (*figs.* 3*m* and 3*bm*), the tongue cell and the two lower cells which surround its base. A section of the apex is circular in outline and consists of the tongue cell alone (*fig.* 3*a*). The three-celled stage of the conceptacle is apparently formed occasionally in another way. The two longitudinal sections of an initial cell are shown in *figs.* 4, 5, containing three nuclei but no walls. Two nuclei appear in one section and one in the other. It seems that the nucleus of the initial cell in this instance divided first with its spindle perpendicular to the axis of the cell, and that one of the daughter nuclei divided with its spindle parallel to the axis.

After the three-celled stage, the development of the conceptacle is readily followed. The two lower of the three cells divide longitudinally in various planes. A condition thus results which exhibits five cells in longitudinal median section (*fig.* 6). Four of the five cells are young cells of the recent divisions, and one is the centrally placed tongue cell. Longitudinal divisions continue as before, and a structure showing six or seven cells in longitudinal section is formed (*fig.* 7). The tongue cell is still conspicuous in this and in several succeeding stages. Longitudinal divisions continue as illustrated in *figs.* 8, 9, 11, until the walls of the entire conceptacle are formed. Some of the wall cells begin to develop sexual organs when the conceptacle is very small (*figs.* 9, 11). This activity of the cells, however, does not prevent them from contributing to the growth of the conceptacle. The mouth of the conceptacle is surrounded by a marginal ring of epidermal tissue about one or two cells deep (*figs.* 8, 11). As these

cells are not aggressive they may be omitted from further consideration. Excluding this minor detail every portion of the conceptacle is the product of one initial cell. Cortical tissue adjacent to the initial takes no part in its development.

The behavior of the tongue cell is similar to that of the "initial cell" in other forms as reported by BOWER. It may show signs of degeneration (*fig. 8*), may remain inactive for some time (*fig. 11*), or may even divide to form a filament of two or three cells (*figs. 9, 10*). In no case does it contribute to the walls of the conceptacle, but on the contrary after its divisions resembles a paraphysis. The tongue cell is very conspicuous until sexual organs begin to develop, but shortly after their appearance it cannot be distinguished. The upper and lower cells which result from the transverse division of the initial cell (*fig. 2*) correspond in appearance and behavior with the "initial cell" and "basal cell" as described by BOWER and others. It seems probable that BOWER saw both the initial cell and the two-celled stage of the conceptacle, but failing to observe the division in the initial cell, considered the initial and the upper cell of the two-celled stage identical. With this construction, degeneration of the upper or tongue cell was believed to be degeneration of the initial cell itself, and the division of the lower cell of the two-celled stage, a product of the initial cell, was regarded merely as the division of an unrelated cortical cell. A conceptacle developed from cells which happen to be adjacent to a degenerating and unimportant cell would be a very different structure from a conceptacle developed from one active initial cell.

THE ORIGIN AND DEVELOPMENT OF THE CRYPTOSTOMA.

The references embodied in the preceding treatment of the conceptacle constitute the chief source of information bearing upon the cryptostoma. The structure which produces the sexual organs has commonly and naturally been given first attention, but investigators who have studied both, generally agree that the conceptacle and cryptostoma are homologous. Different theories regarding the significance of the cryptostoma have been offered, but no safe generalization can be made until a more extended investigation of both structures has been made in a variety of forms.

Miss BARTON ('91) gave an account of the cryptostoma in Turbi-

naria, stating that an initial cell divides longitudinally, thus forming two daughter elements which produce paraphyses. In demonstration of this two paraphyses are figured arising from the base of a many-celled structure. Miss BARTON does not report the origin of the walls of the cryptostoma, but as the initial cell is believed to develop directly into paraphyses, we may assume that she believed the walls to arise from neighboring tissue in accordance with the views of the earlier writers.

The development of the cryptostoma in Sargassum follows step by step the history of the conceptacle. The initial cell arises near the apical cell of a leaf or vegetative branch. Longitudinal and cross sections of this cell (*figs. 12, 12a, m, b*) show the same form and structure as the longitudinal and cross sections of the initial cell of a conceptacle (*figs. 1, 1a, m, b*). The activities of the two initials are also identical. The initial cell of the cryptostoma divides transversely, forming a two-celled structure (*fig. 13*) which is comparable in every way to the two-celled stage of the conceptacle (*fig. 2*), consisting as it does of a tongue cell and a larger lower cell. The lower cell divides longitudinally. A group of three cells then results (*figs. 14, 15*) which is entirely similar to the three-celled stage of a conceptacle (*fig.3*). The two lower cells of this three-celled stage then divide longitudinally in one or more planes, forming a structure which shows four or five cells in longitudinal section (*figs. 6, 17*). The center of this structure and of several which follow is occupied by the conspicuous tongue cell (*figs. 16, 17, 18, 19*). Thus by the continued longitudinal divisions of the products of the lower cell of the two-celled stage, the walls of the entire structure are gradually developed. Paraphyses begin to appear in the cryptostoma (*figs. 18, 20*) as early as do the sexual organs in the conceptacle (*fig. 9*). The activity of the wall cells in producing paraphyses, however, does not interfere with their functioning further in developing the cryptostoma (*fig. 21*). Epidermal cells at the mouth of the cryptostoma form here, as in the conceptacle, a marginal ring one or two cells deep (compare *figs. 8* and *19*). The origin of the true walls of the structure, however, may be traced as in the conceptacle to the lower cell resulting from the transverse division of the initial.

The behavior of the tongue cell in the cryptostoma is similar to

that of the corresponding element in the conceptacle. Occasionally the tongue cell of the cryptostoma may develop a prominent filament (*fig. 20*), which is clearly identical in structure with a typical paraphysis (*fig. 21*). The young conceptacle and cryptostoma are so alike that they can only be distinguished by their respective positions on fruiting branches or on young vegetative structures, until the appearance of sexual organs in the one and paraphyses in the other defines their mature characters.

The development of the paraphysis is interesting for its regularity. A wall cell enlarges, pushing into the cavity of the cryptostoma, and then divides transversely (*figs. 18, 20*). The upper cell produces the paraphysis, whereas the lower functions in the development of the wall. The growth of the paraphysis results from the transverse divisions of the cell next the wall (*figs. 21, 22*), a method of growth termed *trichothallic*. The development of a paraphysis in the cryptostoma of Sargassum is, therefore, characteristically basipetal, as BARTON ('91) found in Turbinaria.

A somewhat advanced paraphysis is composed of three regions. That which adjoins the wall of the cryptostoma consists of the large turgescent meristematically active basal cell (*fig. 22*). The middle region is occupied by six or eight short cells which have so recently been formed that they have not had time to lengthen much. The upper region contains several greatly elongated cells. This region in a mature paraphysis is partly within the cryptostoma and partly without, for fully developed paraphyses extend far beyond the surface of the plant.

A peculiar condition found in many cryptostomata deserves special attention. Structures frequently appear between the paraphyses which seem to bear no relation to them. These are papillae and stalked cells, the former like the papillae which precede male organs in a conceptacle and the latter like the male organs themselves. The stalked cells, although slender and probably always sterile, appear to be spermatocysts no longer functional. This surprising condition is of great interest and importance in relation to the homology and significance of the cryptostoma, a structure formerly believed to contain only paraphyses, but which appears also to have sexual organs or their degenerate representatives. That the cryptostoma and conceptacle

are homologous cannot be doubted, since their origin and early development are identical in all details. The occasional appearance of sterile representatives of sexual organs within the cryptostoma further confirms this view of their relationship and strongly supports the theory of BOWER ('80) that the cryptostoma in the Fucaceae is derived from the conceptacle.

The occurrence of conceptacles in special reproductive branches only, the appearance of cryptostomata in both vegetative and reproductive branches, and the development of representatives of sexual organs within the cryptostomata, suggest a line of evolution from plants bearing conceptacles scattered over leaf and branch structures indiscriminately, to the type now under consideration with localization of the conceptacle upon special branches. Certain branches were set apart to bear conceptacles as the conceptacles in all other parts of the plant body were rendered sterile and thus changed into cryptomostata. The presence of sexual organs or their degenerate representatives within a cryptostoma indicates, according to these views, that the process is not carried to its farthest point in Sargassum.

The production of conceptacles upon small special branches only, instead of upon the entire plant, naturally results in fewer conceptacles upon one plant. The conceptacles, however, are much more closely placed than the cryptostomata. On account of their comparatively small size the initials and young conceptacles occupy very little space in the apex of a branch, but farther down on the receptacle the bulging bowls of the developing flask-shaped conceptacles require more and more space, until the mature structures nearly fill the interior of the receptacle and there is only enough intervening tissue to hold the conceptacles together. The cryptostomata, on the other hand, are well scattered upon vegetative branches and mature leaves. The contrast in the placement of cryptostomata with that of conceptacles is, therefore, very marked.

THE SPERMATOCYST.

The male sexual organs (antheridia), which will be called spermatocysts in this paper, according to the terminology of DAVIS (:04), develop from wall cells of the conceptacle in Sargassum as in other forms of the Fucaceae. A wall cell puts forth a papilla (*fig. 23*) which

is cut off by a transverse wall (*fig. 24*). The lower cell becomes a part of the wall occupying the place of the cell from which it arose. The upper cell enlarges for a time and then divides, forming the sperm mother-cell or spermatocyst and its stalk (*fig. 24*, at the right.) A stalk cell may have no other relation than that which it bears to the spermatocyst which it supports, or it may function in other ways. It may produce several spermatocysts directly, without individual stalks; it may put forth a papilla which gives rise to a spermatocyst and stalk (*figs. 25, 26*); or it may develop a hair (*fig. 27*). Hairs, however, are comparatively rare within a conceptacle of Sargassum. Owing to the variety of activities which belong to a stalk cell, the growths within a conceptacle lack uniformity. Some structures reach but a little distance above the wall of the conceptacle, whereas others form conspicuous branch systems. Although these systems are prominent in this conceptacle, they are considerably smaller and less dense than the branch systems in a conceptacle of Fucus, and there is far more unoccupied space within the cavity of a conceptacle of Sargassum than of Fucus.

The young spermatocyst contains dense cytoplasm, a centrally placed nucleus and deeply staining granules, the nucleus remaining in a resting condition for a long period. The divisions of the nucleus were not studied in detail. Sixty-four sperms are apparently formed (*figs. 27* and *28*), agreeing, therefore, with the count announced by BEHRENS ('86) for *Fucus vesiculosus*. The sperms within the spermatocyst have an elliptical outline, a cytoplasmic ground mass, and a somewhat spirally arranged band, which is probably the nucleus. The discharge of sperms was not seen, but a rent, partly terminal and partly lateral in empty spermatocysts, indicates their mode of escape.

THE OOCYST.

The female sexual organ (oogonium) or oocyst, according to the terminology of DAVIS (:04), is peculiar among the Fucaceae, as far as is known, in that it is not borne upon a stalk but is a partially embedded organ (*fig. 31*). The sister cell of the oocyst, instead of developing into a pedicel cell as is usual in this family, functions as one of the wall cells of the conceptacle. The oocyst enlarges greatly, but becomes nearly surrounded by adjacent wall cells.

Its development is simple. A somewhat enlarged wall cell of a young conceptacle divides transversely, forming two cells much alike in size and contents (*fig. 29*). The inner cell, which is the homologue of the stalk cell of the female organ in Fucus, cannot be distinguished from neighboring wall cells shortly after its formation. The outer cell, which has a free surface toward the interior of the conceptacle, increases greatly in size and soon becomes the spherical oocyst. *Fig. 30* represents a young oocyst and its sister cell, already unequal in size. There now follows a long period of growth, during which the oocyst attains a remarkable size, finally containing a great quantity of reserve material, many chromatophores, much cytoplasm, and a large nucleus. The mature organ, drawn under a lower magnification than *fig. 30*, is represented in *fig. 31*. No trace of its sister cell could be found.

The oocyst of Sargassum develops but one egg. The mitosis within the wall cell whose division produces the oocyst is normally the only mitosis in the process of oogenesis. Particular attention was given to this point. The one nucleus of the oocyst remains in a resting condition throughout the entire period of the growth of the cell, and therefore becomes the nucleus of the egg. In the other genera of the Fucaceae, as is well known, there are three mitoses within the oocyst, resulting in eight nuclei. Each of the eight nuclei may become a center for the development of an egg as in Fucus, or some nuclei may degenerate and a less number of eggs be formed, as in Ascophyllum and Pelvetia. It might be supposed from these conditions in the Fucaceae that the oocyst of Sargassum would show similar nuclear divisions and degeneration, but this is not the case. The mitoses characteristic of oogenesis in Fucus are normally suppressed in Sargassum. The tendency in the Fucaceae to reduce the number of eggs produced by an oocyst reaches its culmination, therefore, in Sargassum.

It is interesting to note that Sargassum still gives proof that it belongs to the reduction series which has its beginning in Fucus and allied forms that produce eight eggs in an oocyst. Out of the great number of conceptacles examined, one oocyst was formed which contained two eggs, and two oocysts which contained eight. The oocyst with two eggs was formed in an immature conceptacle that held five normal oocysts. The two eggs appeared fairly vigorous. One of the

two oocysts which contained eight eggs was an old conceptacle, from which other sexual elements had apparently long been discharged. The eight together were smaller than one normal mature egg. The other oocyst which contained eight eggs shared a conceptacle with two normal oocysts. It was attached in the side of a conceptacle near the surface of the plant, which for a slight distance was modified in structure as if in response to an injury. It is possible in this case that the wound incited the reversion. The appearance of an oocyst containing more than one egg in Sargassum must be regarded as a rare reversion to the Fucus type.

The resting nucleus of the oocyst is always large, but varies in structure. Sometimes it has few granules and no conspicuous reticulum, whereas at other times it contains many granules and a dense network. The nucleolus is also large in size and variable in structure. At the present time no suggestion can be made to account for the changes in nuclear structure, excepting that they are the concomitants of growth and varying nutritive conditions.

The method of discharge of the egg from the conceptacle of Sargassum is somewhat unlike that reported in Fucus and other genera. In Fucus the outer membrane of the oocyst remains attached to the conceptacle, as explained by THURET, and the eggs escape in a group surrounded by a very delicate inner membrane. In Sargassum the entire oocyst becomes freed from the conceptacle and escapes. In Fucus the inner membrane dissolves or breaks, thereby freeing the naked eggs which it has enclosed. In Sargassum the wall of the oocyst swells, stretches, and sometimes ruptures, but it may persist for a long time, even enveloping later a many-celled sporeling formed within it. The inner membrane enclosing the eggs of Pelvetia is separated from the outer as in Fucus. In Pelvetia, however, as figured by THURET, this membrane persists about the eggs, apparently offering no great resistance to the entrance of sperms. Whether the sperm enters the egg of Sargassum through a break in the oocyst membrane, whether it passes through the membrane, or whether the eggs develop parthenogenetically, is not known. A study of fertilization in Sargassum is surrounded by serious technical difficulties because both eggs and sperms develop upon the same plant, thus making it difficult to isolate the sexual cell.

THE SPORELING.

Many if not all of the eggs of Sargassum on leaving the conceptacle become fastened immediately by the mucilaginous wall of the oocyst, which still surrounds it, to the surface of the reproductive branch. In this position the eggs segment. The first division of the egg in Sargassum does not differentiate a rhizoidal region, as in Fucus and Ascophyllum. Instead, a many-celled ellipsoidal structure is formed, the divisions occurring with mathematical precision. Rhizoids then develop at one end with no apparent relation to a substratum or to gravity, so far as could be observed in fixed material. Sporelings sometimes occur at opposite sides of a branch with rhizoids directed toward the stem, thus showing no relation in the development of rhizoids to gravity; and again, sporelings occur with rhizoids directed away from the branch in various directions, indicating that the parent plant exerts no special influence. It is possible that the attachment of a sporeling upon a plant is so insecure that the direction of its axis may be shifted in the manipulation of material. Otherwise it is difficult to account for the conditions which were observed.

When the many-celled sporeling has reached the condition for rhizoid formation the cells at one pole elongate, thereby giving rise to a tuft of rhizoids of approximately equal length. This mass of rhizoidal filaments finally produces the characteristic disk-shaped holdfast of the mature plant. *Fig. 32* shows a sporeling in about the oldest condition in which it remains attached to the parent plant. No apical cells were found in these sporelings and therefore its differentiation must occur after the sporeling has separated from the parent plant.

The germination of the oospore deserves careful cytological investigation. Many preparations have been made and studied, but further attention will be given the subject before the observations are published. A few conditions may be noted, however. There are numerous radiations at the poles of the early spindles. The asters contain granular inclusions which suggest centrosomes, although their origin and relation to the processes of mitosis have not been traced. Walls following the mitoses are developed somewhat slowly, being formed in part at least by the membranes of contiguous vacuoles.

SUMMARY.

Each stem, branch, and leaf structure develops through the activities of a three-sided apical cell.

The thallus consists of three compact tissues, called for convenience the epidermal, cortical, and conducting tissues. The latter consists of only thin-walled cells in the leaves, but in mature stems contains both thick and thin-walled elements. A ring of thick-walled cells, which may have both a mechanical and conducting function, surrounds the thin-walled conducting cells in the center of the axis.

The tissues normally contain much reserve material, a part of which is oil, and a part, whose nature is undetermined, appears to be a carbohydrate.

Both the conceptacles and cryptostomata originate in a single flask-shaped initial cell which develops the entire structure.

The first division of the initial cell results in two unlike segments: a large lower cell which develops the walls of the conceptacle and cryptostoma; and an upper cell, the tongue cell, which either remains inactive, divides to form a short filament, or degenerates. The "initial cell" of BOWER is apparently the tongue cell, a product of the true initial cell.

The conceptacle and cryptostoma are undoubtedly homologous structures. Every stage of development in both structures is the same, from the appearance of the similar initial cells to the development of paraphyses in the cryptostomata and sexual organs in the conceptacle.

The paraphyses are developed basipetally by the division of the lowermost cell in each structure.

Spermatocysts or their degenerate representatives occur in some cryptostomata. Such conditions indicate that the cryptostomata have been derived from conceptacles whose sexual organs have become sterile.

The spermatocysts develop as in other Fucaceae, each finally producing sixty-four sperms which are discharged from a partly terminal and partly lateral rent.

The sister cell of an oocyst does not become a stalk and consequently the oocyst is an embedded structure.

The oocyst normally gives rise to but one egg. The nucleus of the oocyst accordingly becomes the nucleus of the egg.

The oocysts were found containing eight eggs each. These must be considered a rare reversion to the Fucus type.

The entire oocyst of Sargassum, unlike other genera of the Fucaceae which have been studied, is discharged with its enclosed egg. The oocyst wall may break, partially freeing the egg, or it may persist even enveloping a many-celled sporeling.

Segmentation of the egg takes place while it is attached to the surface of the plant by the mucilaginous wall which surrounds it. This segmentation results first in a many-celled undifferentiated ellipsoidal sporeling. Rhizoids develop late at one end of the multicellular sporeling, with no apparent relation to gravity or other stimulus.

Asters, containing granular inclusions suggesting centrosomes, appear at the poles of the spindles in the early mitoses of the segmentation of the egg.

THE UNIVERSITY OF CHICAGO.

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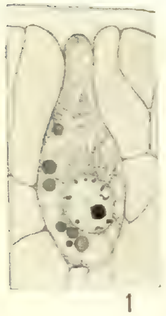
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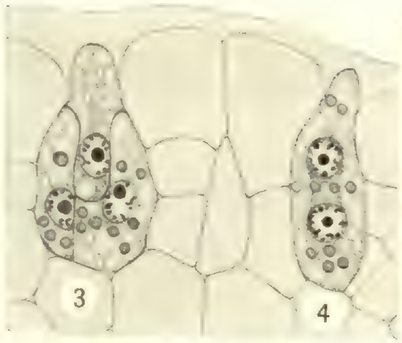
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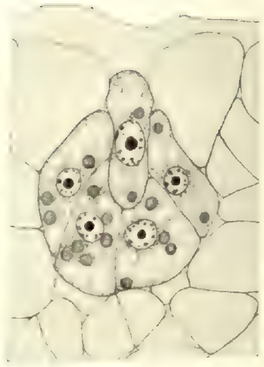
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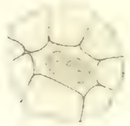
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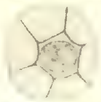
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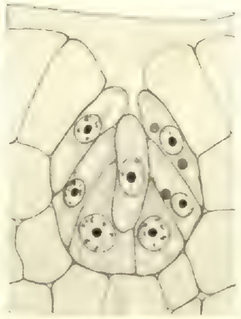
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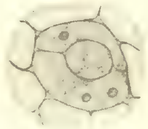
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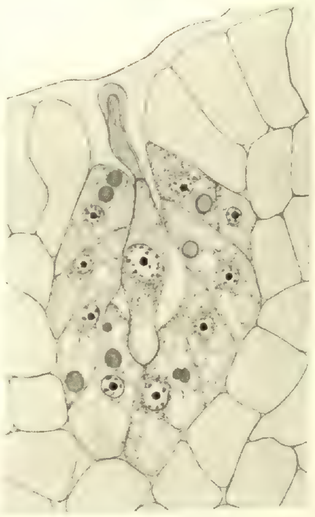
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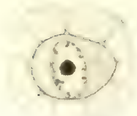
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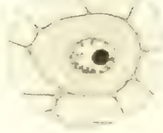
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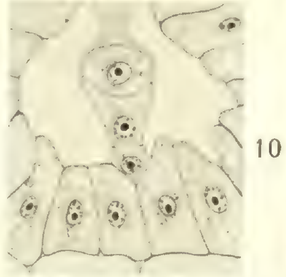
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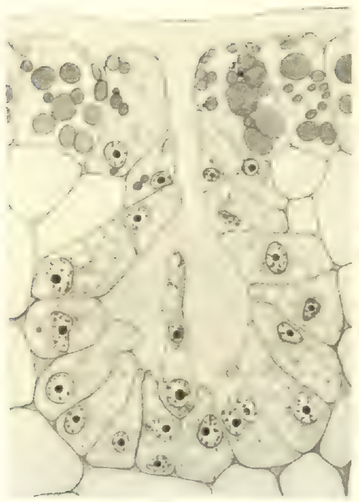
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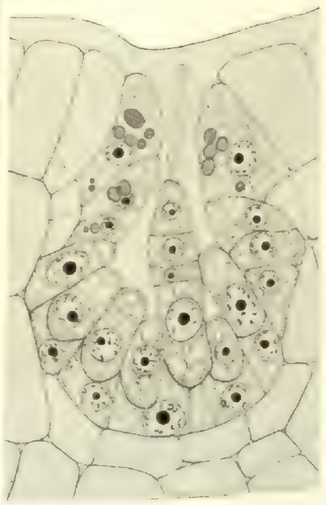
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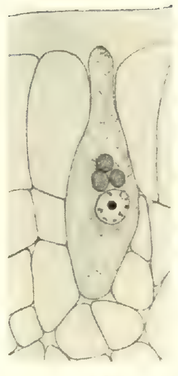
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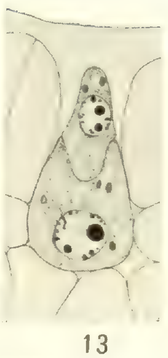
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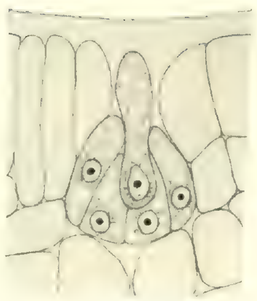
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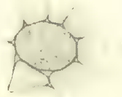
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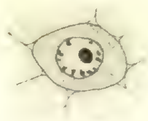
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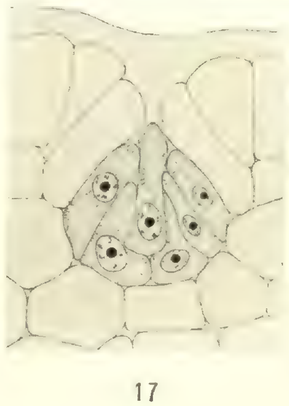
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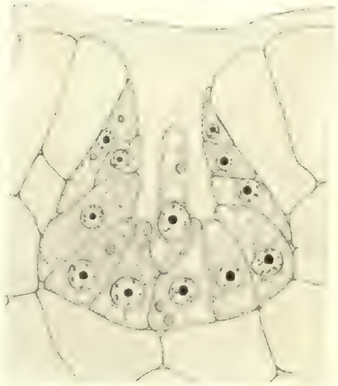
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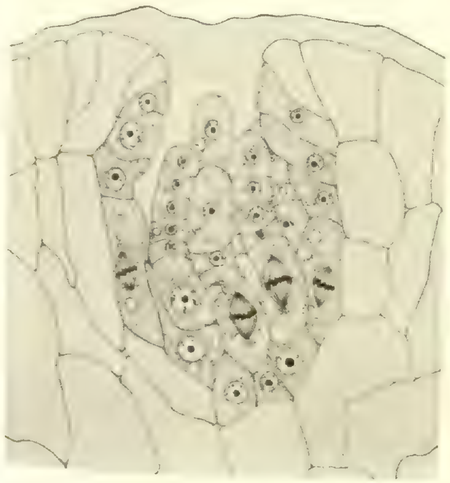
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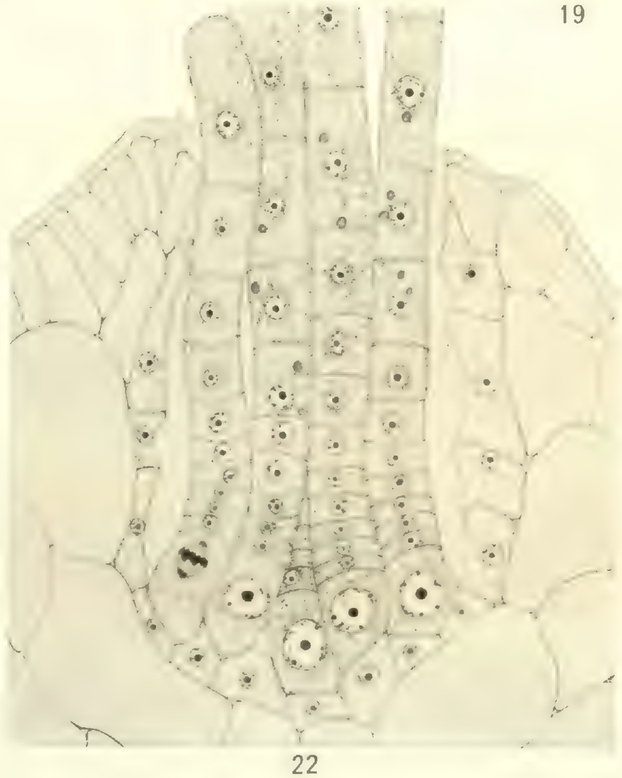
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Etoile B. Simons, del.

SIMONS on SARGASSUM

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EXPLANATION OF PLATES X AND XI.

All figures were sketched with a camera and reduced one third in reproduction. *Figs. 1–30* were drawn with Zeiss apochromatic objective 1.5^{mm} and compensating ocular number 4, magnification 1140. *Figs. 31* and *32* were drawn with dry objective, magnification 570.

PLATE X.

Figures 1–11. Development of the conceptacle.

FIG. 1. Initial cells, longitudinal section; *1a*, cross section of apex; *1m*, cross section of median portion; *1b*, cross section of basal portion.

FIG. 2. Two-celled stage, longitudinal section showing the slender upper tongue cell, and a larger lower cell; *2a*, cross section of the apex showing the tongue cell only; *2m*, cross section of the median portion with the centrally placed basal region of the tongue cell surrounded by the upper part of the lower cell; *2b*, cross section of the basal portion showing the lower cell only.

FIG. 3. Three-celled stage, longitudinal section; *3a*, cross section of the apex showing tongue cell only; *3m*, cross section of median portion with the centrally placed lower part of the tongue cell, surrounded by the upper part of its two companion cells; *3bm*, cross section a little below *3m*, showing the same cells; *3b*, cross section of the basal portion showing the two lower cells only.

FIGS. 4 and 5. Longitudinal sections of a peculiar trinucleate stage of one conceptacle. The first division of the nucleus of the initial cell must have been with the axis of the spindle perpendicular to that of the cell. *Fig. 5* contains one of the nuclei of the first mitosis and *fig. 4* the products of a division, now in late telophase, of the other nucleus of the first mitosis.

FIG. 6. Longitudinal section of a young conceptacle showing four walls cell and the tongue cell.

FIG. 7. Longitudinal section of a later stage with six similar wall cells and the centrally placed tongue cell.

FIG. 8. Longitudinal section of a more advanced stage illustrating the formation of the cavity of the conceptacle.

FIG. 9. Longitudinal section of a young conceptacle some of whose wall cells are developing papillae. The tongue cell contains two nuclei.

FIG. 10. A filament of three cells formed from the tongue cell.

FIG. 11. Young conceptacle showing simultaneous development of wall cells and papillae.

Figures 12–22. Development of the cryptostoma.

FIG. 12. Initials, longitudinal section; 12*a*, cross section of the apex; 12*m*, cross section of median portion; 12*b*, cross section of basal portion.

FIG. 13. Longitudinal section of the two-celled stage.

FIG. 14. Longitudinal section of the lateral surface of the three-celled stage.

FIG. 15. Longitudinal section of the interior of the same group of cells represented in *fig. 14*.

FIG. 16. Longitudinal section showing four wall cells and the tongue cell.

FIG. 17. Longitudinal section slightly more advanced.

FIG. 18. Longitudinal section of a young cryptostoma beginning to form paraphyses very early.

FIG. 19. Longitudinal section of an older stage which has not yet begun to develop paraphyses.

FIG. 20. Longitudinal section showing five paraphyses developing from wall cells and one from the tongue cell.

FIG. 21. More advanced, illustrating the simultaneous development of paraphyses and wall cells.

FIG. 22. Still more advanced.

PLATE XI.

Fig. 23. The development of papillae which will later give rise to spermatocysts.

FIG. 24. At the left a cell which results from the separation of a papilla from a wall cell. At the right a spermatocyst and stalk which have been formed by the division of a cell similar to the one shown at the left.

FIG. 25. A stalk cell has given rise to a papilla, now separated by a wall.

FIG. 26. A branch system formed through the activity of stalk cells.

FIG. 27. A spermatocyst containing sperms. The stalk cell has developed a hair.

FIG. 28. A mature spermatocyst, the stalk cell pushing out at one side.

FIG. 29. Very young oocyst with its sister cell, which is the homologue of the stalk cell in Fucus.

FIG. 30. Slightly older oocyst and its sister cell already unequal in size.

FIG. 31. A mature embedded oocyst containing many chromatophores and much reserve material.

FIG. 32. A sporeling still attached to the surface of the parent plant. At one pole rhizoids have begun to develop. The old wall of the oocyst surrounds the sporeling.

CHROMOSOME REDUCTION IN THE MICROSPOROCYTES OF LILIUM TIGRINUM.[1]

JOHN H. SCHAFFNER.

(WITH PLATES XII AND XIII)

THE progress recently made in our knowledge of hybrids has given a new impetus to the study of chromosome reduction. Unfortunately, there is still much disagreement in the accounts of various observers. In order to continue my own investigations on a very favorable object, the microsporocytes of *Lilium tigrinum* were selected, since material is easily obtained in large quantities. CHAMBERLAIN has studied the pollen grain of this plant and has also given figures of the microsporocyte in the spirem stage. The chromatin granules are exceptionally distinct, and this facilitates the correct interpretation of the complex figures to be seen in the reduction karyokinesis.

Recently papers on the reduction division have been published by FARMER and MOORE, STRASBURGER, MONTGOMERY, WALLACE, and others, which are in essential agreement with the interpretations of DIXON on *Lilium longiflorum* and my own observations on *Lilium Philadelphicum* and Erythronium. On the other hand BERGHS, GRÉGOIRE, and ALLEN have come to somewhat different conclusions. The observations of ROSENBERG on Drosera have opened up an important field of investigation on the individuality of the chromosome. These papers have been reviewed so recently by various writers that it is needless to discuss the results here. It is sufficient to say that it must appear to an impartial judge that the cytologist is at present able to see in his preparations almost anything which may be conceived of as taking place in the structures investigated. This, however, should not hinder work in such an important field, for the proper interpretation can be attained only by continued observation. Little can be regarded as certain until there is a more general agreement among competent investigators. So far as the present research is concerned, the extent and variety of the preparations

[1] Contributions from the Botanical Laboratory of Ohio State University. XXIV.

studied seem to preclude the possibility of mistake. Such doubts as were expressed in my former papers have been practically removed. So far as the writer is concerned, the interpretation given below is conclusive. Another investigator might perhaps come to different conclusions by using other methods.

MATERIALS AND METHODS.

Stamens of various ages were collected in Clay County, Kansas, during July 1904; killed in weak chrom-acetic acid (chromic acid 0.3^{gm}, glacial acetic acid 0.7^{cc}, water 99^{cc}), passed gradually through the grades and preserved in 70 per cent. alcohol, imbedded in paraffin, cut 10–18 μ thick, and stained on the slide. Some old slides, the material of which had been killed in the ordinary chrom-acetic acid solution, were also at hand. Various stains and combinations were used, but for bringing out the chromatin network and chromatin granules of the early stages, Delafield's haematoxylin, when properly developed, gave by far the best results, being superior in this respect to either Heidenhain's iron-alum-haematoxylin or safranin and gentian violet. Nucleoli, both in the nuclear cavity and in the cytoplasm, are stained very distinctly by the safranin-gentian-violet combination, but are only slightly affected by Delafield's haematoxylin.

I am indebted to my wife, MABEL SCHAFFNER, for the preparation of most of the two hundred serial slides on which the present paper is based.

INVESTIGATION.

Before the microsporocytes are beginning to separate the prominent chromatin network is being transformed into slender delicate threads. These threads appear to be discontinuous in some places. However, the appearance may be due to injury during the process of cutting. The threads are small in diameter as compared with the single chain of spherical chromatin granules (*figs. 1–3*). After the spirem becomes fully developed it shows no free ends and is much wound, looped, and twisted. In this stage it appears to be entirely free in the nuclear cavity and is usually in the so-called synapsis stage. Sometimes the contraction is to one side of the nuclear cavity, sometimes near the center, but often little or no contraction is evident. Whether this is an artifact or a real stage in the process of karyokin-

esis appears still to be an open question. Such contractions are so easily produced by the ordinary killing reagents used, and have been described for such a variety of the early stages of nuclear division, that it seems to me no importance is to be attached to observations which have been made on killed material. So far as any opinion is to be expressed upon the appearances in *Lilium tigrinum*, I am still convinced that the extraordinary distortions commonly figured owe their origin to the action of the fluids used before imbedding in paraffin.

The spirem remains simple during the entire synapsis. The linin thread becomes thickened and the chromatin granules are usually more or less elongated (*figs. 4, 5*). At this stage the spirem has already a strong tendency to be thrown into loops and coils (*fig. 4*). After the microsporocytes have become partly separated and more spherical, they were rarely observed to be in synapsis. The nuclear cavity enlarges and the chromatin ribbon becomes thicker, with the granules still more prominent (*figs. 6–8*). This is an important point to consider, for we have here a clear case of sporocytes, long past the supposed synapsis stage, showing with remarkable clearness a simple continuous spirem, with a single chain of chromatin granules.

Synapsis, therefore, can have nothing to do in this case with a supposed longitudinal conjugation of two spirems or two networks of chromatin before the spirem is formed. After the nuclei have passed on to the stage represented in *fig. 6*, the stages are so easily followed and the threads so prominent, that a longitudinal conjugation, if one occurred, could not escape notice. Shortly after the stage shown in *fig. 6* the spirem begins to show double rows of elongated chromatin granules, but the relative quantity of chromatin ribbon present in the nuclear cavity is not much diminished (*figs. 9, 10*). If the amount of spirem were diminished one-half by a longitudinal conjugation the fact would certainly be noticeable. Often a part of the spirem appears still single, or it will appear with a double row of granules and gradually change to a row apparently single (*figs. 11–17*). The appearance would be the same whether the granules were dividing or conjugating. The uniformity of the pairs of granules on the linin thread is remarkable, and the pairs themselves suggest a division. If a conjugation of the chromatin granules were established, it would certainly show

an almost inconceivable regularity. The granules would have to be definite and fixed, of the same number in both the egg and sperm, and not be subject to increase or diminution (*figs. 14–16*). In the early stages of the spirem there are, of course, numerous instances of threads lying parallel, side by side, but these appearances are equally common after the chromatin granules appear double (*figs. 15, 17*).

The ribbon now begins to show an arrangement into definite loops (*fig. 10*). It becomes much shorter and thicker (*figs. 18, 19*) and finally shows a definite twisting together into twelve loops, which have their heads toward the nuclear wall (*fig. 20*). At this stage the chromatin granules can still be distinguished lying side by side (*fig. 21*). But at this time the whole ribbon begins to undergo a change, so that it stains of a uniform, dense color throughout, and before the loops separate all evidence of chromatin granules is lost (*figs. 22, 23*). That the loops shown in *figs. 18–23* are the incipient chromosomes is self-evident. By no manner of interpretation can such a conclusion be explained away. By the time the twelve loops have separated, the nucleoli have entirely disappeared from the nuclear cavity (*fig. 24*). The nucleoli break up into micronucleoli and are thrown out into the cytoplasm (*figs. 38, 51*). The figures in which they do not appear were taken from material stained in such a way that the nucleoli were not evident or not very distinct.

The chromosomes are exceedingly interesting on account of the many fantastic shapes produced by the coiling of the ribbon. A series of distinct shapes is given in *figs. 25–37*. Occasionally the loop shows evidence of its double nature (*fig. 29*), but usually it appears homogeneous throughout. Much time was spent in a study of these chromosomes, and the variety of shape and coil could be extended indefinitely. In all cases the chromosome is continuous, the outer end of the loop always being closed. Occasionally the coiling takes place in such a way as to form a double loop (*figs. 26, 27, 35*). The chromosome is situated on the spindle with its head or closed end outward (*figs. 28–30*). Sometimes it is very difficult to unravel the nature of the coil, as in *fig. 35*. In other chromosomes it is an easy matter to follow out the details of the loop, as in *fig. 28*.

The spindle threads are evidently attached some distance back of the free limbs of the loop (*figs. 25–28*). The limbs are gradually

uncoiled and pulled apart. They are separated at the closed outer end. A transverse splitting of the chromosome is thus accomplished. There is no evidence that the two limbs of the chromosome are a male and female chromosome joined end to end and twisted together until they make a longitudinal pair. But theoretically such a proposition is easily possible or even probable. Because the spindle threads are attached some distance from the ends of the limbs, the daughter chromosomes are developed as V- or U-shaped loops (*figs. 40, 43, 51*). In favorably stained sections some evidence of chromatin granules may be observed. These still show a distinct pairing in some cases (*fig. 44*), but in others the arrangement is considerably disturbed (*fig. 45*). The daughter chromosomes, as they appear in the daughter star, are of various forms and are sometimes twisted (*figs. 44–50*).

The micronucleoli are gradually collected below the two daughter skeins, and are finally all inclosed in the nuclear cavities of the daughter nuclei (*figs. 52, 53*). The daughter chromosomes do not appear to form a very definite resting network, but are transformed into the mother skein of the second division rather rapidly. Whether a continuous ribbon is formed was not ascertained. The loops are already separate at an early stage, and it is possible that the daughter chromosomes of the first division, after forming an imperfect reticulum, break up directly into the twelve chromosomes of the second division (*fig. 54*). This point, however, is doubtful. But the absence of a definite resting stage in sections having microsporocytes with loose daughter skeins in close proximity to loose mother skeins of the second division, gives support to the above supposition. The micronucleoli are again distributed in the cytoplasm before the mother-star stage of the second division (*figs. 54, 55*). The karyokinetic figures of the second division are easily distinguishable from those of the first. This is especially true of the mother star (*figs. 38, 55*). The chromosomes in the second division have their free ends directed outwards, as in an ordinary vegetative division. Commonly they are more or less tangential to the spindle. Sometimes, however, they stand at right angles, as represented in *figs. 56, 57*.

One of the most difficult points to determine was the nature of the splitting in the second division. However, it was definitely ascertained that the splitting is longitudinal. Dividing chromosomes are

represented in *figs. 58–61*. The daughter loops are completely separated very early in their migration towards the poles (*fig. 62*). They are also of various shapes. Frequently straight chromosomes are present in the daughter star and very commonly the J shape predominates (*figs. 63, 64*). U and V shapes are also present. After the chromosomes have passed into the daughter skein stage they show an irregular outline with a row of irregular chromatin granules (*figs. 65, 66*). The chromosomes develop into irregular networks, showing the remains of the original loops, and thus pass into the spore tetrad stage (*fig. 67*).

During the early germination stages of the microspores the figures are again remarkable for the large nucleoli in the cytoplasm (*figs. 68–70*). From the appearance of the nuclei during the several divisions, it is evident that the nucleoli do not contribute directly to the formation of the chromosomes, but that they are uniformly thrown out into the cytoplasm, in a fragmented condition, during the earlier stages of karyokinesis.

No study of the achromatic structures was attempted, and though some interesting points were observed from time to time, there was nothing which calls for special mention.

SUMMARY.

1. The first division of the microsporocyte is a true reduction division.

2. A continuous spirem is formed with a single row of chromatin granules.

3. The spirem passes through and comes out of synapsis without a conjugation or division of chromatin granules.

4. The chromatin granules divide but the linin thread does not show a distinct separation.

5. The continuous spirem shortens and thickens and twists up into twelve loops, which are the incepts of the twelve separate chromosomes.

6. The chromosomes are arranged in the mother star with the loop or head end turned outwards and the spindle threads are attached near the ends of the free limbs or about half way between the free ends and the head.

7. During the metakinesis stage the chromosomes uncoil and separate by a transverse division at the middle.

8. The chromosomes of the second division appear to represent the daughter chromosomes of the first division.

9. The division of the chromosomes in the second nuclear division is longitudinal.

10. The nucleoli fragment and pass out into the cytoplasm during the first and second divisions and also during the germination of the microspore.

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EXPLANATION OF PLATES XII AND XIII.

The plates are reduced five-eighths in reproduction. The figures representing entire cells and nuclei were studied with a Leitz no. 4 ocular and $\frac{1}{16}$ oil immersion objective; the others with a Zeiss no. 12 ocular and a Leitz $\frac{1}{16}$ oil immersion objective.

PLATE XII.

FIG. 1. Microsporocyte with chromatin network beginning to form the spirem.

FIG. 2. Microsporocyte with delicate threads showing a single row of prominent chromatin granules.

FIG. 3. Single thread of the same.

FIG. 4. Sporocytes beginning to separate. Nucleus with distinct continuous, contracted spirem in the so-called synapsis stage.

FIG. 5. Single thread of the same showing a single row of chromatin granules.

FIG. 6. Sporocyte some time after the synapsis stage, showing prominent spirem with single row of chromatin granules. The free ends are cut.

FIGS. 7, 8. Single threads of the same.

FIG. 9. Sporocyte with chromatin granules mostly double and flattened in appearance.

FIG. 10. Somewhat later stage, showing the beginning of looping of spirem, and division of chromatin granules. One nucleolus beyond the nuclear cavity.

FIGS. 11–17. Single threads from the same stage, showing chromatin granules still single, some with granules partly double, and others with typical double rows of granules.

FIG. 18. Microsporocyte with loops nearly developed.

FIG. 19. A single loop of the same stage.

FIG. 20. Chromatin loops some time before separation; somewhat cut.

FIG. 21. A single loop from the same stage.

FIG. 22. Section of microsporocyte, showing chromatin loops near the time of separation.

FIG. 23. A single loop from the same nucleus.

FIG. 24. Microsporocyte, showing the twelve chromosomes.

FIGS. 25–37. Individual chromosomes, showing various types of loops and coils and their position on the spindle threads.

PLATE XIII.

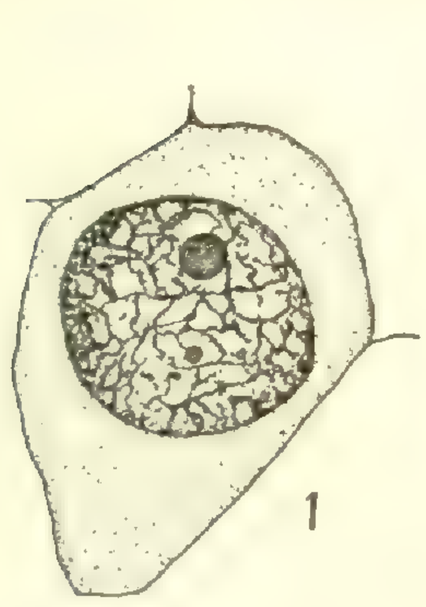
FIG. 38. Mother star, showing position of chromosomes. Micronucleoli in the cytoplasm.

FIGS. 39–42. Chromosomes from mother-star stage, showing mode of separation of limbs of loops.

FIG. 43. Microsporocyte with daughter stars.

FIGS. 44–50. Daughter chromosomes from the daughter star, showing character of of the loops.

FIG. 51. Daughter-star stage, with micronucleoli in the cytoplasm.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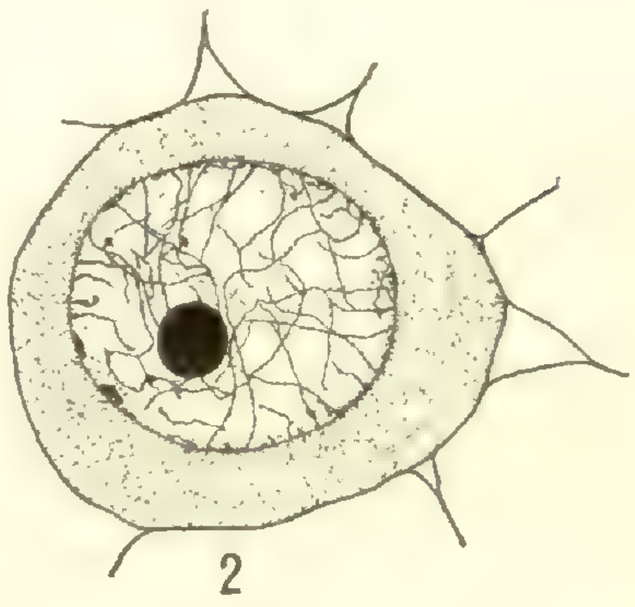
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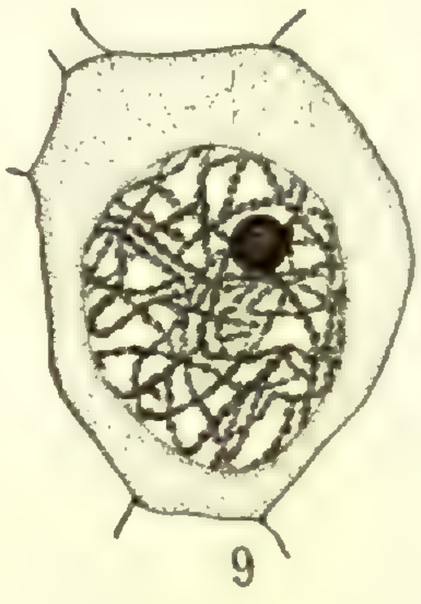
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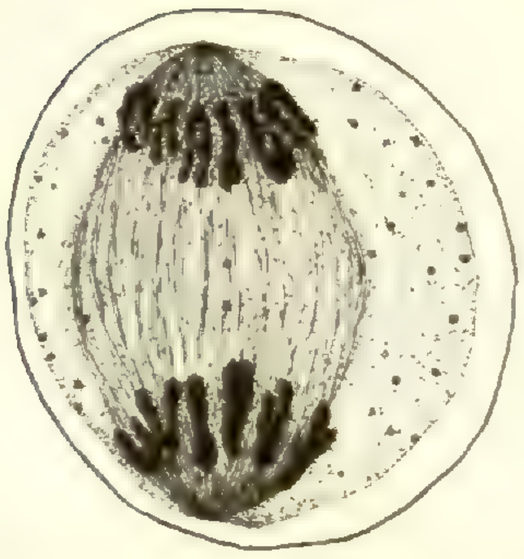
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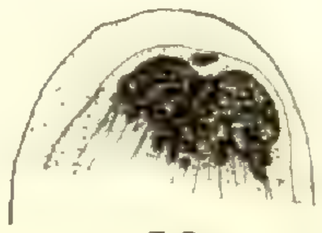
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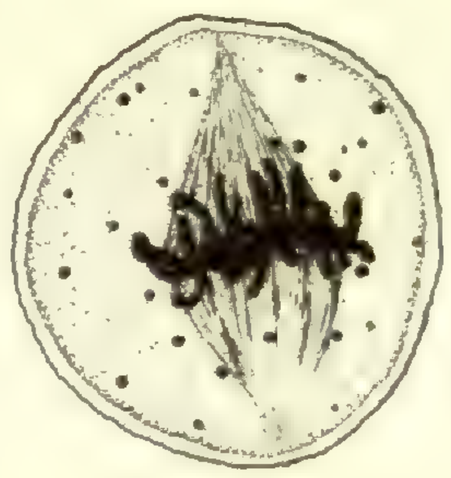
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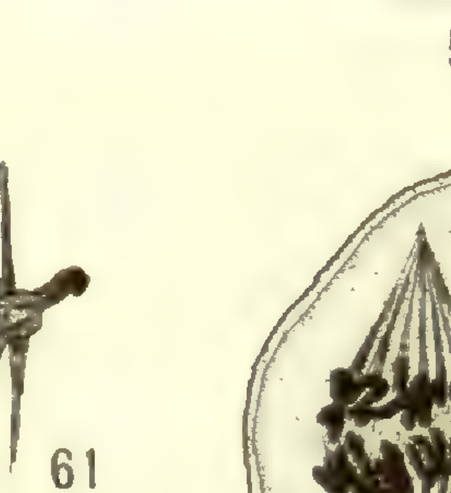
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FIG. 52. Loose daughter-skein stage with micronucleoli collected below the open ends of the chromosomes.

FIG. 53. End of loose daughter-skein stage, with micronucleoli collected among the chromosomes.

FIG. 54. Beginning of second division, with chromatin loops in the nucleus and micronucleoli in the cytoplasm.

FIG. 55. Mother star of the second division, showing characteristic appearance of the chromosomes, with micronucleoli in the cytoplasm.

FIGS. 56, 57. Single chromosomes from the mother-star stage, showing position on the spindle threads.

FIGS. 58–61. Chromosomes from the metakinesis stage, showing the nature of the longitudinal splitting.

FIG. 62. End of metakinesis stage.

FIG. 63. Daughter-star stage of second division.

FIG. 64. A pair of daughter chromosomes from the daughter-star stage of the second division.

FIGS. 65, 66. Pieces of chromosomes at the end of the second division, showing a single row of irregular chromatin granules.

FIG. 67. Tetrad at end of second division.

FIG. 68. Microspore at beginning of germination, with two large nucleoli in the cytoplasm.

FIG. 69. Same stage as the preceding, showing three nucleoli in the cytoplasm.

FIG. 70. Microspore in germination stage, showing a number of nucleoli in the cytoplasm.

CYTOLOGICAL STUDIES ON THE ENTOMOPHTHOREAE.

I. THE MORPHOLOGY AND DEVELOPMENT OF EMPUSA.[1]

EDGAR W. OLIVE.

(WITH PLATES XIV AND XV)

THE general development of the Entomophthoreae and the external morphology of its various members have been studied by a number of investigators. For a detailed review of the literature pertaining to the group, the reader is referred particularly to the papers of BREFELD ('71,'81,'84) and of THAXTER ('88).

COHN'S ('55) results in his classical paper on the developmental history of *Empusa muscae* have been in certain respects considerably modified by later investigation. According to him, the fly first became diseased and the fungus followed as a consequence. The first indications of the disease which COHN could find in the blood of the fly were numberless minute globular or irregularly shaped bodies, whose presence he could not explain otherwise than by the assumption of spontaneous generation (p. 334).

These bodies, according to him, grow larger, become globular or ellipsoidal, and finally grow into the filament, which, by the formation of partitions, becomes the three-celled hypha characteristic of the mature fungus and consisting of spore, stalk-cell, and root-cell. This three-celled character of the hyphae of *Empusa muscae* was disproved, however, the next year by LEBERT ('56) and later by BREFELD and others.

Every investigator who has attempted to infect insects artificially has testified to the difficulties which he has encountered. COHN ('55, p. 342), in speaking of his own lack of success, wisely emphasizes the caution which should characterize such experiments, noting that one should be certain that the insects experimented with are not already stricken with the disease, a more difficult task than would at first appear.

[1] I am under obligation to the Carnegie Institution of Washington for grants, which have rendered possible this investigation.

BREFELD ('70, '71, '77), however, was successful in transmitting the parasite through external inoculation of spores, and he found that in the case of *Empusa muscae* infection took place only through the thinner whitish parts of the skin on the under side of the fly's body; whereas in another species, *E. sphaerosperma*, the infected hyphae gained an entrance at any part of the skin of the cabbage larva. This author has contributed more on this point than any other, in that he was able to germinate the conidia in artificial media as well as on the surface of the insect body, and to find with the microscope the germinating hyphae actually boring through the skin of the host. In his series of articles, BREFELD has described the complete course of development of two species of Empusa, *E. muscae* Cohn and *E. sphaerosperma* Fres., which furnish quite different types of vegetative growth. According to him, in *E. muscae* there are formed from those germ-tubes which have penetrated into the body-cavity of the insect numerous detached non-nucleate cells, which reproduce by repeated yeast-like sprouting, and which grow within the fat-bodies of the host. At a certain advanced stage of the development of the fungus, the reproduction of the cells by budding ceases, and each grows at one or both ends into a long unbranched tube, which grows through the body-wall and produces at its external end a single conidium. In the other species, *E. sphaerosperma*, BREFELD found that the germ-tube produces, on the other hand, a copiously branching mycelium with many cross-partitions, which finally fills the body-cavity of the host. At the end of the vegetative period, this mycelium sends out hyphae which grow to the surface, branch digitately, and finally produce acrogenously at each ultimate end a single conidium.

Empusa sphaerosperma further differs from *E. muscae* in producing resting spores. COHN ('55, p. 343) had already suggested, since he could not make the conidiospores of *E. muscae* germinate, that perhaps the conidia themselves required a year of rest. But BREFELD ('70, '71), proved conclusively that the spores of this form were short-lived, living only for a few days; hence his first suggestion in regard to the puzzling question as to the wintering of such a species was that this form was probably heteroecious, and that resting spores were produced in some other host. Later, however ('84, p. 68), he seems

inclined to believe that the disease is continued over winter on flies in warmer regions, and that it migrates northward with the insects on the return of summer; the fallacy of which theory THAXTER has pointed out.

THAXTER ('88) in his account of the Entomophthoreae of the United States, gives the results of morphological studies based on a considerable number of new as well as old forms. This author disagrees with BREFELD in regard to certain important points. In the first place, he maintains that the vegetative growth in *E. sphaerosperma* is not filamentous in all cases, as is stated by BREFELD, and he appears to be inclined to think that both the filamentous mycelium and the broken-up, budding segments may occur in the same form under different conditions. He asserts that the usual multiplication of the hyphae is not by branching and continuous growth but by the formation of "hyphal bodies," which "consist of short thick fragments, of very varied size and shape, that are continually reproduced by budding or division, until the insect is more or less completely filled with them." But he continues further: "In cases where a direct mycelial growth follows the entrance of the hypha of germination, if indeed such instances occur, this mycelium must fall to pieces into hyphal bodies, before the commencement of growth the direct object of which is reproduction, in a fashion resembling that above described at a similar stage for Conidiobolus" (p. 140). This conception of reproduction by means of "hyphal bodies," however, for reasons that are stated later in this paper, must be abandoned, at least as a generalization.

The segments of the vegetative hyphae, or "hyphal bodies" as THAXTER terms them, under unfavorable conditions may each form a thick-walled resting chlamydospore; or, when the conditions are favorable, they may at once proceed to develop into the fructifying state. In the latter case, according to this author, each hyphal segment sends out one or two (in some species more) hyphae which develop into conidiophores. In the simplest case, a simple conidiophore grows directly to the outer air and produces a single conidium (*Empusa muscae*, e. g.). Or, the conidiophore may become compound and produce a set of conidia. Or, under very favorable conditions, "a single primary hypha may branch indefinitely, each

ultimate branch becoming a conidiophore similar to those of the more simple case just mentioned" (p. 142). A singular method of germination of the "hyphal bodies" occurs in *E. aphidis* and *E. virescens*, according to THAXTER'S observations. Spherical bodies, evidently regarded as "hyphal bodies" with highly refractive contents, germinate and send out a mass of hyphae in all directions. In this condition they are said to resemble a head of Aspergillus, although the author does not show in either of his two drawings of the phenomenon any trace or remnant of the central cells or "hyphal bodies" from which the radiating hyphae are said to arise.

The conidium is regarded by THAXTER as having a double wall, and thus is to be interpreted more properly as a simple single-spored sporangium.

MATERIALS AND METHODS.

In March 1904, the writer found in horse-dung cultures in the laboratory a small species of fly belonging to the genus *Sciara*, which was infected with an Empusa. This small fly with its attendant disease has been propagated in horse-dung cultures since that time, and many successive generations of the insect, the larval condition as well as the adult, during the year and more of its cultivation, have furnished a wealth of material for an almost complete cytological and developmental study of this species of Empusa. A number of other forms of the Entomophthoreae, most of them in the fructifying stage of their existence, have been used for comparison, but no others have as yet been traced through their entire life history. Enough has been learned, however, to show the existence of a most interesting series of distinctive variations.

My material has been killed with a variety of fixing agents, mostly with varying strengths of Flemming's chromic-acetic-osmic acid mixture. The insect body was generally cut in two or pricked to allow direct contact of the fixing fluid and the fungus hyphae in the body cavity. The material was sectioned usually 3–6 μ thick and stained with Flemming's safranin gentian-violet orange-G, or with Heidenhain's iron haematoxylin.

Six species of Empusa altogether have been thus studied. These species, determined according to the descriptions in THAXTER'S

account of the group, are as follows: *Empusa muscae* Cohn, on the common house fly; *E. culicis*, A. Braun, on a small green species of Chironomus; *Empusa* sp., on a large fly; *E. aphidis* Hoffman, on various aphides; *E. americana* Thaxter, on a blue-bottle fly; and one other species, which is the principal one studied in this paper, on the small fly, *Sciara* sp. The ovoid conidia of this last form coincide closely with the description of the conidia of *E. montana* Thaxter. They show decided differences, however, from THAXTER's drawings of this species; and from *E. ovispora* Nowakowski and *E. echinospora* Thaxter, with which the measurements of the conidia also almost coincide, the form is distinguished by the characteristic zygospores of the latter species. While it is possible that, under certain conditions unknown to me, this species on Sciara may produce zygospores, yet the fact remains that after over a year of continuous observation, I have failed to find any resting spores. It is therefore thought advisable to give a new name provisionally to this species, which will hereafter be referred to as *Empusa sciarae*.

Empusa sciarae n. sp.—Vegetative hyphae forming a branching, septate mycelium, which in advanced stages is cut up into few- (generally 3–5-) nucleate cells. Radial hyphae branched; conidiophores 3–5, bearing at each ultimate end a single ovoid uninucleate conidium with a rounded basal papilla, 12–16 × 18–25 μ. Zygospores unknown.

VEGETATIVE STAGE.

My own observations of purely vegetative stages are concerned with two forms only, *Empusa aphidis* and *E. sciarae*, both of which agree with the type described by BREFELD for *E. sphaerosperma*. In all the other species studied, the vegetative condition had ceased and conidiophores had grown out from the vegetative hyphae. As observed by BREFELD, THAXTER, and others, the insect dies at the end of the nutritive period of the fungus; but they do not seem to have emphasized the fact that living insects alone must furnish data for the study of the vegetative hyphae. After the initiation of the reproductive period and the consequent death of the insect, the radial growth of the conidiophores produces a mass of hyphae which might readily be taken, in certain instances, for a vegetative filamentous mycelial growth. The probability has suggested itself to the writer

that the fat cells, detached in the search for the fungus, must have been mistaken by some for stages in the development of Empusa; since these bodies may frequently resemble closely short hyphal segments in their fatty, granular contents, as well as in their assumption of globular or irregular shapes, which give the suggestion of budding cells. These detached fat-bodies, which prove in sections to be small aggregates of insect cells, are particularly abundant in the adult just preceding ovipositing. An easy and certain method of distinguishing the vegetative cells of Empusa from fat-bodies which occur with them in the body cavity, is to stain with a dilute methyl-green solution, acidulated with a few drops of acetic acid, when the fungus cells stand out conspicuously, distinguished by their relatively large and characteristic nuclei.

In some preliminary observations on the manner of infection of *Empusa sciarae*, I have not been able to make certain of this point, but I wish to record here some notes of interest pertaining to it. The small fly, *Sciara* sp., was sometimes accompanied, in the vessels in which the dung cultures were kept, by three other species of small flies, which hatched generally in less abundance, *Psychoda* sp., a so-called moth fly, and two other undetermined forms. None, however, other than Sciara were infected, although many times I have noted conidia stuck on the surface of the bodies of the larvae as well as of the adults of the other species. Successive generations of infected Sciara larvae as well as adults have appeared with great regularity every month or six weeks. The fly lays its eggs on the surface of the dung or on the sides of the vessels, and the young larvae, soon after hatching, crawl below the surface of the substratum. It would appear reasonable to assume that in this case infection would occur with ease in this very young condition, when the larval skin is thin and delicate, and before they had crawled below the surface of the dung, where they would obviously not be reached by shooting conidia. My preliminary unsuccessful experiments at infecting healthy adult larvae, confined for a week in bottles with diseased ones, at least suggest the possibility of infection occurring in young insects only. If the cultures in which young larvae were being nourished were kept quite moist, perhaps the majority of individuals in this stage were killed by the disease. When, however, the conditions were

drier, the insect developed frequently into the adult fly, which itself, after ovipositing, generally died of the disease.

In order to determine whether the disease could be carried from larval to adult condition, I have examined many pupae, but only in one or two instances have hyphae been found. In one case in which a young insect was struggling to get out of its pupal shell, a few Empusa hyphae were noted when the body was cut open, together with many small eggs and globular fat-bodies. When cultures were kept at some distance from infected ones, in an adjoining room, Sciara frequently developed to maturity by hundreds and died finally a natural death, evidently without infection.

Resting spores in this species, as in *E. muscae*, have not been observed; hence the puzzling question confronts us here also as to the method of wintering of the fungus. An interesting suggestion is made in this connection by the spontaneous appearance of *Empusa sciarae* in laboratory cultures as early in the spring as March, in 1904. This fact apparently still further renders useless BREFELD'S hypothesis as to the migration of the fungus north from warmer countries. The host, in this case, must have been breeding in the dung of the warm stables all through the winter, and it is quite reasonable to suppose that the short-lived fungus must have been continued at the same time on successive generations of insects. The successful cultivation of the form for more than a year in the laboratory gives additional reason for this belief. It is possible that *Empusa muscae* and such similar forms may have lost their sexual stage, because of their success in propagating the disease by means of conidia alone. It is well known that a few house-flies survive the winter by hibernation or otherwise, and it is probable that some of the individuals during the winter may continue breeding in stables or in other favorable places, and in this way carry over the disease, even in cold climates.

Living larvae of Sciara furnish beautiful material for a study of the vegetative conditions of this type of Empusa. When placed on a slide in water, a glance with the naked eye is sufficient to determine whether the larva is infected or not, since the diseased individuals are whitish in appearance, due to the presence of the more or less copious mycelium, while uninfected ones are transparent. Under the microscope, the diseased larvae show clearly the long, branched, mycelial

filaments, which in a young condition may have but few septa, while in a later vegetative stage they develop numerous septa. As the larvae crawl about over the surface of the slide, the hyphae can be seen sometimes extending the full length of the body-cavity, sometimes copiously developed in the posterior portion only, again more abundant in the anterior region. As the body parts move, or the blood flows in the cavity, the mycelium shows corresponding pulsations and movements. *Fig. 1* shows a portion of a branched vegetative hypha, taken from such a larva which was crawling just below the surface of the dung culture. Evidently the vegetative activities have here almost ceased, and the 2-, 3-, or 4-nucleate cells are about ready to send out the radial conidiophores. *Fig. 2* shows a section of a younger hypha, in which the cells contain a varying number of nuclei; while *fig. 3* represents a still younger stage, the earliest condition within the body of the host which I have succeeded in obtaining, growing in a larva in which the mycelium was composed of but a few scattered filaments. The section of the hypha, of which *fig. 3* represents only a portion, shows in the preparation twenty-two nuclei, but in the entire length, as far as traceable, not a single cross partition.

While in my own investigations of the vegetative stages of *Empusa sciarae* there remains as yet a small gap, from the penetration of the infecting hypha to the production of such multinucleate mycelial filaments as are shown in *fig. 3*, I feel reasonably certain as to the method of procedure. Germinating conidia, growing in a sterilized decoction of cooked larvae, are shown in *figs. 4–9*. *Figs. 4–7* illustrate the germination of the uninucleate conidia into germ-tubes which have grown out of the liquid decoction, thus resulting in the formation at once of secondary conidia; *fig. 7* shows the beginning of the formation of a tertiary conidium. In *figs. 8* and *9* the germ-tube has grown into a hypha in which all of the protoplasm appears to be in the end cells; in the latter, sixteen apparently empty cells separate the old conidial wall from the terminal protoplasm, which still remains uninucleate.

Both BREFELD ('**71**, *figs. 5, 29, 31*) and THAXTER ('**88**, *fig. 240*) give figures showing a somewhat further advance over what I have obtained in the cultivation of conidia, in that in their forms branches

are beginning to appear. Whether an increase of the amount of protoplasm and of the number of nuclei has accompanied this branching is doubtful.

It is highly probable, therefore, that in *Empusa sciarae* the hypha which has penetrated into the body-cavity of the fly from the germinating conidium grows rapidly at the expense of the nutrient fluids in which it floats. After the protoplasm has increased in volume, the nuclei increase in number by division, and from the uninucleate condition, in the case of *Empusa sciarae*, the hypha finally becomes a multinucleate branching filament, such as is shown in *fig. 3*. Partition-walls in this form at first occur but sparingly; later, however, at the culmination of vegetative activity, septa are abundant and branching becomes more frequent. Finally the body-cavity of the insect becomes completely filled with the mycelial filaments, vegetative activity ceases, and the fructifying state begins.

Empusa aphidis furnishes a somewhat modified vegetative development. The advanced condition, which is my only source of information in this case, shows in sections branched coenocytic hyphae, which appear to be but rarely divided by septa, unlike the corresponding stage in *Empusa sciarae*. Even after the rhizoids have grown out from the under side of the body of the insect (*figs. 10, 11*), the vegetative activities appear to continue, as evidenced by the fact that the nuclei in this instance are still undergoing division. Also in *Empusa* sp. there occurs a similar prolongation of vegetative activity, since at an advanced stage even fewer cross-partitions can be found in the coenocytic mycelium (*figs. 23, 25*).

REPRODUCTIVE STAGE.

At the culmination of vegetative growth, the body-cavity of the larva appears to be completely filled with a mass of long hyphae. Toward the close of this stage, the larva crawls to the surface of the substratum or high up on the side of the culture dish; or, in the case of the adult Sciara, the fly seeks a conspicuous position, as is common with such infected insects, and death ensues with the beginning of the fructifying condition.

The initiation of the fructifying condition is marked in *Empusa sciarae* by the sudden formation of radial branches from the short

cells which make up the hyphal filaments. These branches appear to be put forth more or less simultaneously, in this species but one from each cell. The cell swells up and becomes rounded off somewhat at its ends (*fig. 13*). One or more vacuoles appear in the protoplasm and a protuberance is pushed out from one end of the cell; this grows into the radial hypha destined to become the branched conidiophore (*figs. 12, 14, 15*). The two forces, the swelling and consequent rounding off of the cells at the ends and the pushing out of the branch, combine to split the partition-walls between the cells, thus causing the hyphae to become easily broken up into one-celled segments, the "hyphal bodies" of THAXTER (*figs. 1, 12*). I have seen similar hyphal segments, forming in these instances also the origin of the radial conidiophores, as well in my preparations of *E. culicis* and *E. americana;* but other species promise interesting variations from this common type of pre-reproductive development. In *Empusa* sp. and *E. aphidis*, for example, the vegetative hyphae remain, up to the very initiation of the reproductive stage, either unicellular, or at least with cross-partitions at only rare intervals. In such instances, therefore, no breaking up into "hyphal bodies" occurs; but the vegetative hyphae appear to grow out directly into the conidiophores.

As BREFELD and THAXTER have pointed out, the first hyphae to appear in the external growth of the fungus form the rhizoids, by means of which the host is fastened firmly to the substratum. In the common house-fly, the host is attached by means of its proboscis. In *E. sphaerosperma*, according to BREFELD, bundles of rhizoids break out more or less irregularly from the under side of the body of the insect and attach it to the substratum. According to THAXTER, these rhizoidal hyphae may branch, and may terminate in a kind of expanded sucker, which apparently secretes a viscous substance.

In *Empusa sciarae*, rhizoids are developed more or less abundantly from the under side of the abdomen of the fly, or, in the case of the larva, from almost any point on the under side of the body. In certain forms of *E. aphidis*, groups of rhizoids break out from the under surface of the insect and form large sucker-like hold-fasts. In several instances, I have counted three of these hold-fasts from aphides parasitic on Solidago. *Fig. 10* shows one of these sucker-like bundles of rhizoids in section; and *fig. 11* a highly magnified hypha from near

the inner region of the hold-fast. The latter presents clearly the character of the typical rhizoid; thick, frequently yellowish walls, containing but a thin layer of protoplasm, which bounds externally the large vacuolar cavity that almost fills the hypha. The walls of the ordinary vegetative hyphae, on the other hand, are thin; those of the rhizoids in this case appear to have undergone a gelatinous or slimy modification, and the contents seems to be undergoing degeneration.

The growth of the conidiophores, in the case of *E. sciarae*, proceeds more slowly than that of the rhizoidal hyphae, a phenomenon which is probably due, in part at least, to the slower absorption of water by those hyphae destined to bear conidia. At any rate, the vacuoles which are formed at this time in the cells increase slowly in size, and a conidiophore arises from near the end of each cell and grows out radially, boring its way through the tissues of the host (*figs. 12, 14, 15*). As THAXTER and others have noted, the conidiophores of certain species remain simple and unbranched, as in the case of *E. muscae* (*fig. 40*); or, in other species, they may become normally septate and branched, as shown in *E. sciarae* (*fig. 16*), and *Empusa* sp. (*fig. 23*). From this fact arise most interesting cytological variations in the various conidia of these species. THAXTER has brought out clearly the variation in size, shape, etc., of the conidia of many species, but CAVARA ('99) was the first to contrast the multinucleate condition of the conidia of *Empusa muscae* with that of the uninucleate conidia of *Entomophthora Delpiniana*. Of the six species studied by me, four have uninucleate conidia (*E. sciarae*, *fig. 27*; *E. americana*, *figs. 36, 37*; *E. aphidis*, *figs. 42, 43*; and *Empusa* sp., *figs. 22, 26*). The conidia of *E. culicis* are normally two-, rarely three-nucleate (*figs. 31, 32*); while those of *E. muscae* have a more or less indefinite number, frequently about 15–18 (*figs. 38, 39*).[2] The more common uninucleate conidia arise primarily from the septation of the conidiophores into uninucleate segments; whereas, on the other hand, the simple conidiophore of *E. muscae* does not usually become septate except at the conidium. In the last case, therefore, all of the many nuclei of the last-formed vegetative cell, which forms the origin of the

[2] While convinced of the value of CAVARA'S suggestion as to the use of nuclear characters in the classification of the Entomophthoreae I do not think that this one character alone would justify the separation of Entomophthora from Empusa.

conidiophore, flow out into the single, large, bell-shaped conidium (*fig. 39*).

In *E. culicis*, which has binucleate conidia, septation of the conidiophore occurs, by which the protoplasm cuts itself off from behind; but, unlike the case of *E. sciarae*, I have found no branching with it, so that as a consequence, all of the binucleate protoplasm of the conidiophoric hypha flows into the one terminal conidium (*figs. 30–35*). The simple conidiophore of *E. culicis* resembles, therefore, that of *E. muscae* in being unbranched, but the origin of the conidiophores may differ in the two cases. One may find, in fact, in *E. culicis*, two sorts of "hyphal bodies;" either small cells which, like the corresponding ones of *E. muscae*, give rise to but one conidiophore; or, on the other hand, larger cells, which may give rise to several conidiophores by budding from several points simultaneously, in a similar manner to Conidiobolus. In both instances, each conidiophore remains simple and ultimately bears, terminally, the binucleate conidiospore.

In the species with uninucleate conidia, *E. sciarae*, *E. americana*, *E. aphidis*, and *Empusa* sp., the coenocytic conidiophore, as was indicated above, is cut up by septa, in a manner to be described later, into uninucleate segments (*figs. 16–21*). In all these cases, this results in a branching growth and successive abjunction of the acrogenous spores. Below the terminal cell the penultimate cell pushes out to one side, and thence bores its way to the surface of the host, where it abjoints a single uninucleate conidium (*figs. 16, 23*). From this habit in certain forms, arises a profuse system of branching, frequently of a digitate type (e. g., *Empusa* sp.) or corymbose (*E. sciarae* and others), to enable the uninucleate segments to reach the surface and to discharge their protoplasm by means of the abjointed conidia. But the last-formed vegetative cells of *E. sciarae* contain only 2–4 nuclei (*figs. 1, 12, 15*), so that, in this instance, only a correspondingly small number of branches are formed.

The process of abjection of the conidia of Empusa is apparently similar in a general way to that described for the sporangia of Pilobolus, except that in these conidia there is no gelatinous collar visible. The formation of the partition at the base of the conidium in Empusa also is quite a different process from the formation of the columella in the case of Pilobolus.

In Empusa, the vacuole which appears in the basal portion of the

cell becomes larger and larger (*fig. 27*), and a small protuberance, which has a diameter equal to about half that of the conidiophore, is pushed out from the end (*figs. 34, 41*). There is now formed at the apex of this narrowed sterigma a swelling (*figs. 27, 35, 39, 42*), which, after continued enlargement, finally receives the greater part of the protoplasm and all of the nuclear content from the basal portion. The process of cell-division, by means of which this apical conidium is cut off by a wall from the penultimate cell, will be described in some detail later, in connection with the description of cell-division in Empusa. Certain points should be noted here, however, and among them, that the term *basidium*, as applied to the penultimate cell, although in common use in this connection, should, in my opinion, be confined to the Basidiomycetes, where, morphologically, the true basidium is a very different spore-bearing structure from the penultimate cell bearing the conidium in Empusa.

The penultimate cell forms the explosive mechanism by which the conidium is shot off. As the ring-formed wall which cuts through the base of the conidium travels progressively inward, the protoplasm passes through the narrowing opening leading from below, until at the close of abjunction, the basal cell retains only a thin parietal layer of protoplasm, but no nucleus. Continued swelling, due to the absorption of water, finally results in the bursting of the basal vesicle, thereby breaking the wall of the vesicle where it joins that of the conidium. In some forms, a ring-shaped scar is noticeable near the base of the conidium, marking the circle where the summit of the swollen basal vesicle was formerly attached. The septum which separates the conidium from the subterminal cell is at first usually pushed upward, thus resembling a columella (*figs. 28, 30, 31, 36*), but when the spore is shot off, this partition-wall reverses its former position, and in the conidium it appears as a prominent papilla (*figs. 29, 37, 43*). When the basal vesicle bursts, its contents are thrown out of the open ruptured end and frequently persists as a slimy covering about the spore, serving in this case, perhaps, the double purpose of protection against excessive evaporation and of sticking the spore to the substratum which it strikes. I have noted that the explosion, in the case of *Empusa sciarae*, sometimes throws the spore a distance of 6^{mm}; while BREFELD has recorded an even greater distance in the case of *E. muscae*, in which the spores are said to be sent as far as $2-3^{cm}$.

I have not yet completely solved satisfactorily to myself the peculiar method of abjection of the spores of my undetermined species of Empusa. I am inclined to think, however, that this method, in certain respects, is unlike that described above. In this form the conidiophores come to the surface, become cut up by septa into uninucleate segments, and proceed to branch profusely (*figs. 23, 25*). The cell terminating each branch pushes out in a peculiar manner. Instead of forming a large basal vesicle as an explosive mechanism in the usual manner, as described above, here the protoplasm appears to cut itself off from behind by means of one or more successively formed walls (*figs. 22, 24, 26*). A minimum of protoplasm seems to be lost in the process, and this cut-off protoplasm soon assumes a peculiar granular appearance. In this condition it is probably dead, for these cut-off cells appear soon to lose their turgescence. It is difficult to conceive of a forcible discharge of the spore in this instance, especially if it be true that the protoplasm of the basal cells is dead and thus incapable, through loss of turgidity, of functioning as an explosive mechanism. The process here rather seems to be that, by means of these successive abjunctions, the uninucleate spores are pushed off with but little force, and that they are probably followed out of the thick, gelatinous wall of the mother hypha by other cells pushed up from below. It may be, however, that further studies on fresh material of this species will change this impression of subterminal proliferation.

Figs. 45 and *46* represent the terminal portion of large hyphae of *E. culicis* which are destined to form resting spores; and *figs. 47* and *48*, two fully formed, thick-walled resting spores. Such hyphae as are shown in the first two figures are distinguished from conidiophores by being much larger, and, further, they contain four or five nuclei, instead of two. I have traced these hyphae in sections back to large "hyphal bodies," but I was unable to follow their complete history. Whether the thick-walled resting spores of this species are therefore true zygospores, or azygospores, as they are termed in THAXTER's monograph, I am not prepared to say. I have not been able, however, to confirm VUILLEMIN'S assertions (:**00**) as to the nuclear fusions in the azygospores of *Entomophthora gloeospora*.

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EXPLANATION OF PLATES XIV AND XV.

The drawings were made with the aid of an Abbé camera lucida, and for the most part with various compensating oculars combined with Zeiss 2^{mm} apochromatic obj. N. A. 1.30.

PLATE XIV.

FIGS. 1–9, *Empusa sciarae.*

FIG. 1. A freshly killed filament, fixed and stained with acetic methyl green. ×275.

FIG. 2. Section of a younger filament.

FIG. 3. Section of a still younger vegetative hypha. ×275.

FIG. 4. A germinating conidium, cultivated in a decoction of cooked larvæ, forming a secondary conidium. Killed in acetic methyl green. ×575.

FIGS. 5, 6. Conidia with still younger germ-tubes. Killed with acetic methyl green. ×575.

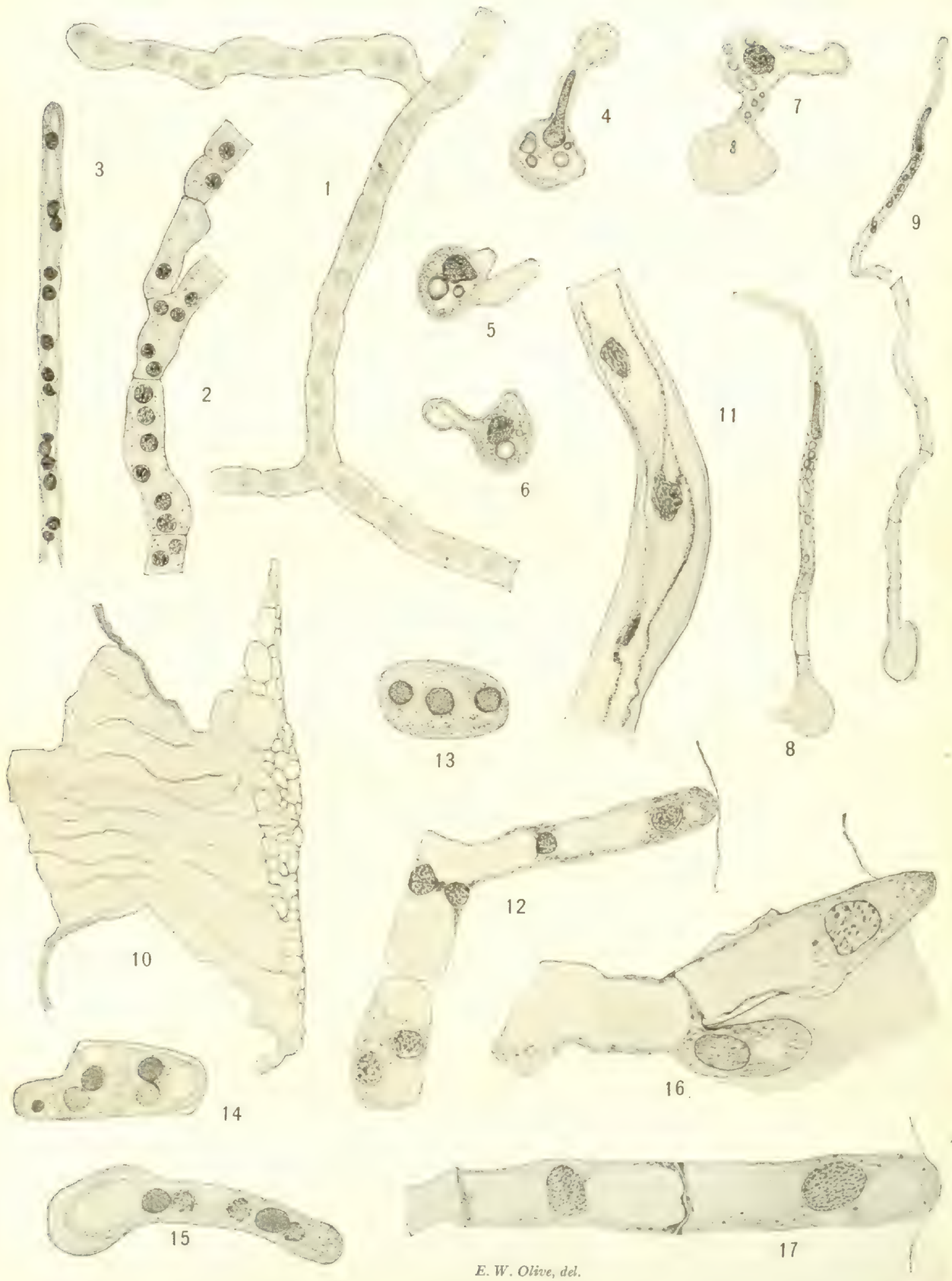
FIG. 7. A germ-tube has formed a secondary conidium, which in turn has started to form a tertiary conidium. Killed ditto. ×575.

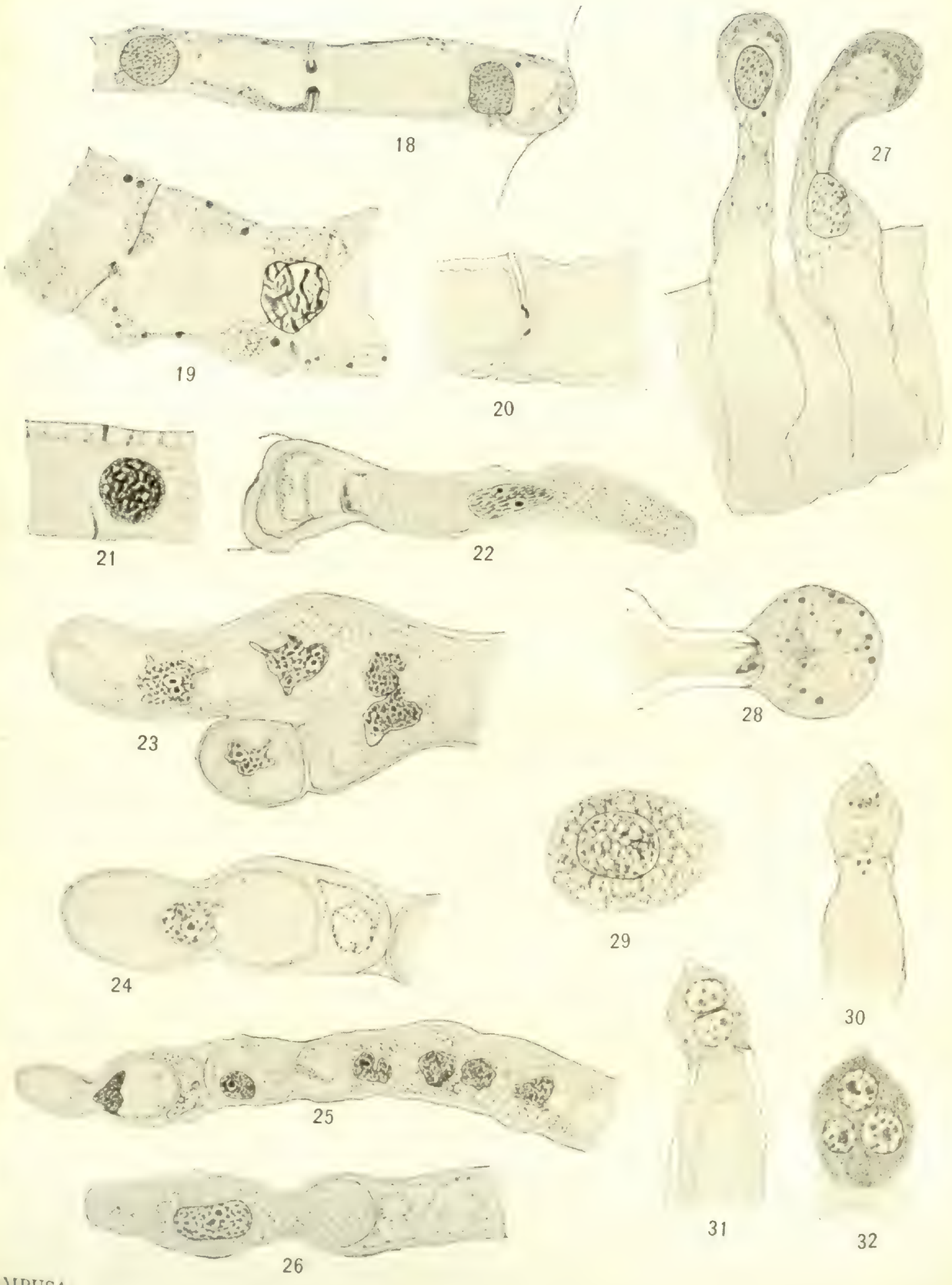
FIGS. 8, 9. The conidia have produced long germ-tubes instead of secondary conidia. From the same culture as those above. Killed ditto.

FIGS. 10–11, *Empusa aphidis.*

FIG. 10. A cross-section of one of the sucker-like rhizoidal disks.

FIG. 11. A section of a single rhizoid. ×1080.





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OLIVE on EMPUSA

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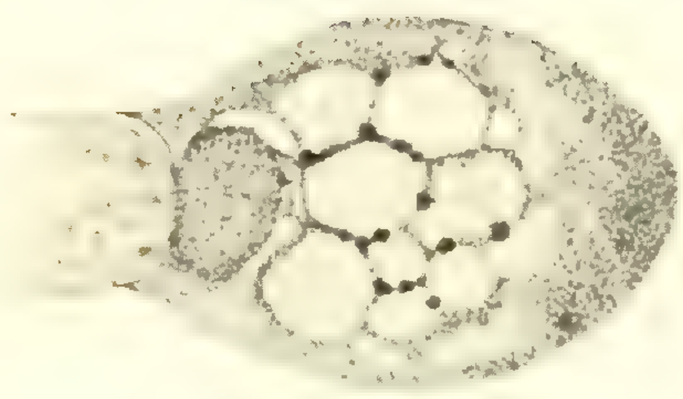

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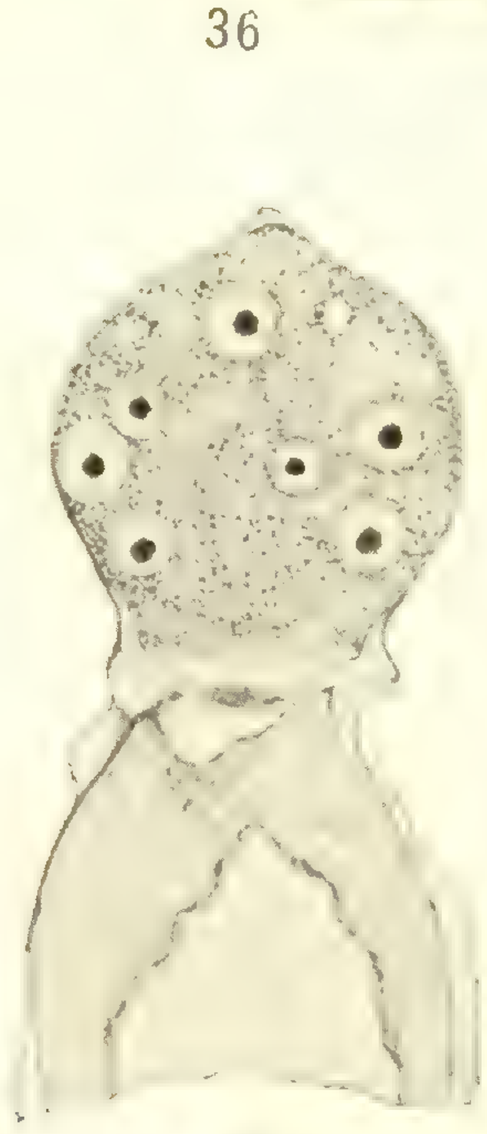

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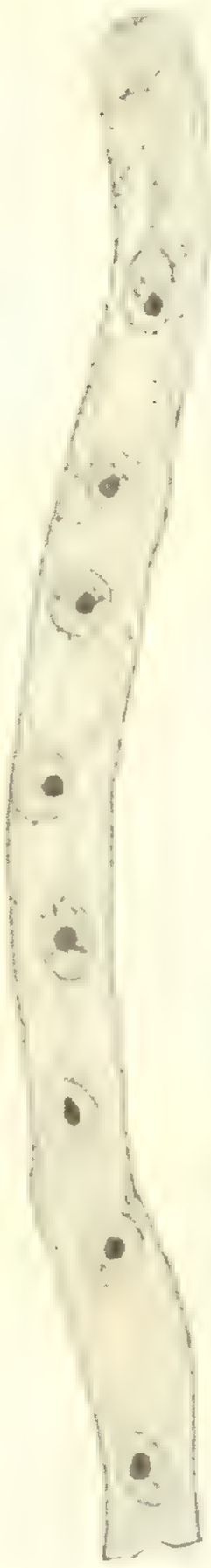

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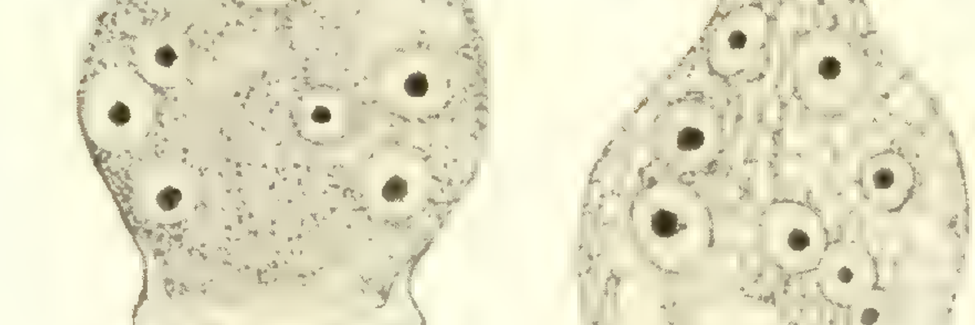

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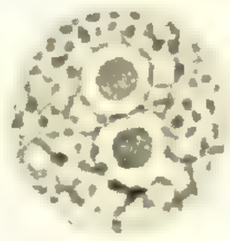

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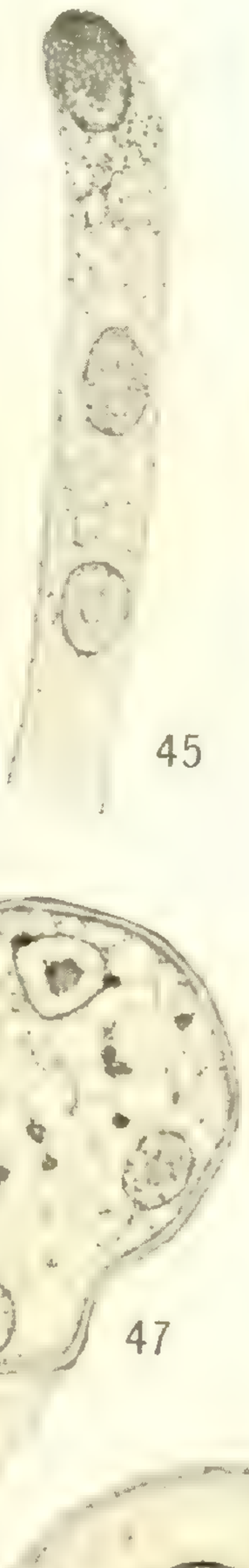

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OLIVE on EMPUSA

FIGS. 12–21, *Empusa sciarae.*

FIG. 12. A section showing two cells of the old vegetative mycelium, or "hyphal bodies," from the upper one of which has grown out radially a conidiophore.

FIG. 13. A hyphal segment freshly killed with acetic methyl green. ×480.

FIG. 14. The same, showing the beginning of a conidiophore. ×480.

FIG. 15. The same, showing a still longer conidiophore. ×480.

FIG. 16. Section of a radial conidiophore which is just emerging from the epidermis of the host, showing the subterminal branching. ×1080.

FIG. 17. Section of a conidiophore showing a completed septum between the two cells. ×1080.

FIG. 18. A half-completed stage of cell-division in a conidiophore. ×1080.

FIG. 19. Cell-division in the vegetative mycelium. ×1500.

FIG. 20. An almost-completed stage of cell-division in a conidiophore; the iron-haematoxylin stain has been almost completely washed out of the preparation, except at the innermost margin of the cleft. ×1500.

FIG. 21. A poorly differentiated construction in a vegetative hypha.

FIGS. 22–24, *Empusa* sp. ?

FIG. 22. Section of a conidiophore, showing the peculiar method of the cutting off of basal cells, which apparently soon die. ×1080.

FIG. 23. Section of a conidiophore showing a subterminal branch, and also nuclei which appear to have assumed amoeboid shapes. ×1080.

FIG. 24. A similar section to that shown in fig. 22, but with only one basal cell cut off. ×1080.

FIGS. 25, 26, *Empusa* sp.

FIG. 25. End of a conidiophore, from which has been cut off two uninucleate segments. ×1080.

FIG. 26. A conidium which is apparently being pushed out of the thick, slimy wall of the mother hypha. ×575.

FIGS. 27–29, *Empusa sciarae.*

FIG. 27. A section showing two protruded terminal cells of the conidiophore, from which young conidia are in process of formation. ×1080.

FIG. 28. A section of a conidium in process of abstriction, in which the iron-haematoxylin stain has not differentiated the nucleus but has brought out some of the metachromatic bodies. ×1500.

FIG. 29. A mature conidium.

FIGS. 30–32, *Empusa culicis.* ×1500.

FIG. 30. The upper portion of the terminal cell of a conidiophore, showing at either side the cleft marking the ring-formed cleavage-furrow.

FIG. 31. A section showing a somewhat older stage, in which the cleft has almost abstricted the binucleate conidium.

FIG. 32. A rare occurrence, showing a 3-nucleate conidium.

PLATE XV.

FIGS. 33–35. *Empusa culicis.* ×1500.

FIG. 33. A thin, lightly stained section of the end of a conidiophore, showing the usual binucleate condition.

FIG. 34. An older stage, in which the sterigma is pushing out.

FIG. 35. From a still older preparation, in which the end of the sterigma has become swollen to form the binucleate conidium.

FIGS. 36, 37, *Empusa americana.* ×1500.

FIG. 36. A preparation in which the nucleus is poorly differentiated, but which shows especially clearly the vacuolar cytoplasm and the columella-like wall which finally abstricts the conidium.

FIG. 37. A mature conidiospore, showing the vacuolated cytoplasm (probably filled when living with oil-globules), the single nucleus, and the basal papilla which has been formed by the reversal of the columella.

FIGS. 38–40, *Empusa muscae.* ×1080.

FIG. 38. The partition-wall which cuts off the multinucleate conidium is here completed. The slimy enucleate protoplasm of the basal cell appears to be but little shrunken.

FIG. 39. The end of a young conidiophore, just extruded from the body of the fly, in which the conidium is in process of formation, showing the multinucleate character of the protoplasm.

FIG. 40. A portion of a conidiophore still within the body of the fly, which is growing toward an opening in one of the abdominal joints.

FIGS. 41–44, *Empusa aphidis.* ×1500.

FIG. 41. A section showing at the tip of the conidiophore a young conidium in process of formation.

FIG. 42. An older stage of conidium-formation.

FIG. 43. A mature conidium, showing the uninucleate character.

FIG. 44. A nucleus from a conidiophore, showing two nucleoles, and portions of the chromatic thread.

FIGS. 45–48, *Empusa culicis.*

FIG. 45. The end of a young azygosporic hypha. ×1080.

FIG. 46. An older condition, which shows the beginning of the formation of a resting spore. ×1080.

FIG. 47. A mature "azygospore." ×1080.

FIG. 48. A thinner section of a mature "azygospore," showing but one nucleus. Other nuclei lie below and above in the section. ×1500.

BRIEFER ARTICLES

NEW NORMAL APPLIANCES FOR USE IN PLANT PHYSIOLOGY III.[1]

(WITH TWO FIGURES)

In the two preceding articles I described several pieces of apparatus newly devised for educational work in plant physiology and explained the objects I have in view in their development. In brief I aim to provide for each of the principal physiological processes such apparatus as will be accurate in results, convenient in manipulation, and obtainable by purchase from a supply company. The company to which the manufacture has been delegated is the Bausch & Lomb Optical Co., of Rochester, N. Y. In the earlier articles I named the appliances "precision-appliances," which some, though not all of them are; they are, however, more properly *normal appliances*, which I shall henceforth call them.

VI. PHOTOSYNTHOMETER.

No fact in all the physiology of plants is more important, and hence more imperatively demands complete demonstration in botanical education, than the absorption of carbon dioxid by green plants in light, with the equivolumetric release of oxygen. There are simple ways of demonstrating the process in part, and somewhat complicated ways of demonstrating it completely; but hitherto there has been no simple method of demonstrating the entire process in one operation. This is effected, however, by the new photosynthometer described below, and illustrated in the accompanying *fig. 1*. It is called by this name for the reason that it permits photosynthesis (the quantity of the photosynthate being a function of the quantity of the gases absorbed and released) to be measured as well as demonstrated.

The instrument consists essentially of a pear-shaped plant-chamber set in a firm iron base, a graduated measuring tube with a small stop-cock at the upper end, and a connecting stopper furnished with a stop-cock of considerable bore. The total capacity of the apparatus when closed is exactly 102^{cc}, of which the 2^{cc} is for a shoot and 100^{cc} for the gases concerned. The proper amount of shoot is provided by selecting a small-leaved plant and pushing a branch down into a measuring-glass until it displaces exactly 2^{cc}

[1] Continued from BOT. GAZETTE 39: 152. February 1905.

of water; the water-level is then noted on the stem, which is cut at this point under water, the shoot being later, when dried, placed upright in the chamber. (It hardly shows in the figure because of irregular reflections from the surface of the chamber.) Taking advantage of the fact that the shoot will carry on photosynthesis for a time in an atmosphere containing carbon dioxid in demonstrably large amount, even up to 10 per cent. or more, we add some selected percentage of that gas to the apparatus in this way. The measuring tube, with stop-cock closed, is inverted and filled with water of room-temperature, up to a figure of the graduation expressing the selected percentage, for the tube is graduated in cubic centimeters, which are, of course, percentages of the total gas capacity of the apparatus. The stopper is then placed on the tube, and its stop-cock closed; its hollow is filled with water and inverted in a pneumatic trough (or equivalent dish of water), which has been standing in the laboratory long enough to take the temperature of the air. The lower stop-cock is then opened and carbon dioxid from a generator is allowed to enter the tube, either from below or, as is most convenient, through the top of the tube. The admission of the gas may be perfectly controlled by cautious manipulation of the upper stop-cock, which is closed at the moment when the water has been wholly driven out to the bottom of the bore of the lower stop-cock, which point is held exactly at the water-level. The lower stop-cock is now closed, and the combination, which now contains exactly the desired percentage

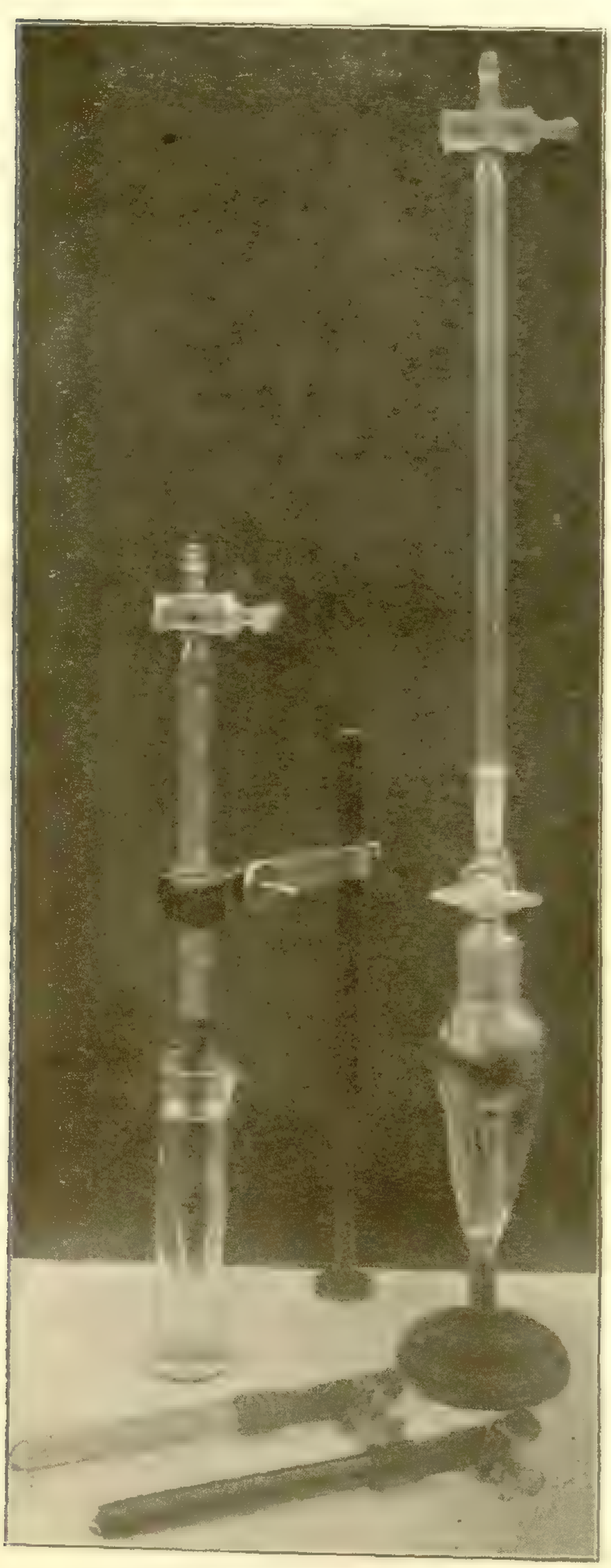

FIG. 1.

of carbon dioxid, is lifted from the water, shaken free from all adhering drops, and placed in position on the chamber. To prevent compression, and therefore the presence of too great a quantity, of air in the chamber when the stopper is pushed into place, tiny holes (visible in the figure), matching in stopper and chamber-neck, allow free release of such pressure, and the chamber is perfectly sealed by twisting the stopper a little. The lower stop-cock is then opened, permitting the carbon dioxid of the tube to diffuse into the chamber, a process hastened by its gravitational flow. The apparatus now contains obviously 2^{cc} of plant and 100^{cc} of gas, of which a known percentage (say 5, 8, or 10) is carbon dioxid, and the remainder is air. The instrument is now placed in a bright light (not direct sunlight) for three or four hours; then the lower stop-cock is closed (as shown in the figure), shutting into the tube a sample of the gas of the chamber at the close of the experiment. The analysis of this gas can be made at leisure, and is accomplished thus. The stopper and tube are removed and placed upright in the pneumatic trough, deeply enough to allow the stopper to be taken off without admission of air to the tube. The zero mark of the tube is then brought exactly to the water surface; the upper stop-cock is cautiously opened, permitting the water to rise slowly to the zero mark, when the stop-cock is again closed, shutting into the tube exactly 10^{cc} of the gas to be analyzed. First the quantity of carbon dioxid in the tube is determined, which is accomplished by aid of a reagent tube, of the form shown at the bottom of the figure. This tube, of glass, is provided with an extension of soft rubber tubing closed by a screw clamp, and it is filled completely to the clamp with a strong solution of caustic potash. It is slipped under the water of the pneumatic trough, with the clamp closed; the air, if any, is squeezed from the upper part; and the rubber is slipped over the lower end of the measuring tube which it grips firmly. The whole is then lifted from the water, the clamp is opened, the combination is inverted and the liquid is allowed to flow back and forth several times from one tube to the other, when it will completely absorb any carbon dioxid present. The clamp is then closed, the combination is slipped again under water, and the rubber tube is pulled off, when the atmospheric pressure will instantly force up the water to the exact extent of the carbon dioxid absorbed, permitting the amount, and hence the percentage, to be read off directly. Next a determination of the percentage of oxygen present is made. This is effected by a precisely similar method, using another reagent tube containing pyrogallate of potash, freshly made up in the usual manner. A few inversions of the combination will result in absorption of all the oxygen, and the water-level in the tube when the rubber is

removed will give directly the percentage originally present. Some slender vessel may then be slipped under the tube which is removed and supported as shown by the figure.

Thus may the gas exchange in photosynthesis be demonstrated accurately, completely, logically, and conveniently.

In studying the process with beginners, the demonstration is the more striking and conclusive to them if a second instrument is set up like (and beside) the first, but covered completely from light; while even a third, like these two except that it has no plant, may advantageously be added. The comparative analyses of the gases at the close of the experiment give results leaving nothing to be desired in logical demonstration.

For all ordinary purposes, water may be used in the pneumatic trough, its slight absorption of carbon dioxid being negligible; but for great accuracy mercury may be employed. Similarly the corrections for capillarity, vapor tension, etc., may for elementary demonstration be ignored, though in precise work they would be taken into account. Temperature and barometric pressure are compensated, obviously, by the method of use of the instrument.

A much larger instrument, capable of taking large leaves, branches, or even entire potted plants, but operated upon substantially the same principle, is now in advanced preparation and will be described later.

FIG. 2.

VII. ALUMINUM SHELLS FOR TRANSPIRATION EXPERIMENTS, ETC.

In transpiration studies with potted plants it is of course necessary to eliminate evaporation from pot and soil. There are many ways of effecting this, of which the best, perhaps, is the use of a tin cup or glass jar to cover the pot, and a roof of rubber sheeting. The advantage of this method over those in which the plant is wrapped in rubber, sealed in melted wax, etc., is this,—that the rubber roof may be readily detached from the can or jar and lifted, permitting a complete change of air to the roots when the plant is watered, thus contributing greatly to the health of the underground parts.

The aluminum shells here figured (*fig.* 2) are designed to provide light, neat, clean, and easily applied covers for pots on this principle. Flower pots are now made so nearly in standard sizes that it is possible to make the shells to fit them closely, and shells will be made for the present in 3-inch, 3½-inch, 4-inch and 5-inch sizes. To hold the rubber roof tightly to the shell, a tightly-fitting band or strap of aluminum, resting in a groove just below the strengthened top of the shell, may be drawn to any desired tightness by a convenient screw-nut, shown (though dimly) on the right in the figure. The rubber roof may be attached to the plant in any of the ways ordinarily used, but I find upon the whole the best method to be the following. In the middle of a proper-sized piece of medium-thick rubber-sheeting, a hole a little smaller than the stem of the plant is made with a cork-borer, and a cut is made with scissors from this to the margin of the piece. It is then placed around the stem, the cut edges of the central hole are stretched to overlap a little, sealed together with rubber cement and held clasped until this sets. Then a line of the cement is run to the margin, sealing one edge over the other. When fully set, the margin of the rubber is clasped to the shell, all surplus material is cut away, and a very neat and perfectly tight roof remains.—W. F. GANONG, *Smith College, Northampton, Mass.*

CURRENT LITERATURE.

BOOK REVIEWS.

Bacterial diseases.

THIS quarto volume on bacterial plant diseases, by DR. ERWIN F. SMITH,[1] is a manual for plant pathologists, which, with the author's characteristic care in clearly stating details, covers the subject, from the angle at which a tube should be held during inoculation to plans for the construction of a water still and suggestions as to the proper developer for photographic plates. One of the fundamenta reasons for the abundance of half-finished work on bacterial plant diseases which is annually imposed upon the public has been the lack in all literature of just such a guide. If the admonitions which the author has scattered through the work were heeded the feeling of uncertainty which now pervades the subject in the minds of many would be promptly dissipated.

The Carnegie Institution is surely treading on dangerous ground in publishing such a manual, but if such action can be justified at all, it can be in this case. Here the need is great and the subject is so specialized as to make such a publication impossible except at a financial loss. So great was the need that a proper presentation of the material promised for the second volume could hardly be made without a considerable portion of the matter here given.

The volume is divided into three parts; 186 pages being devoted to the text, 16 to useful laboratory formulæ, and 63 to bibliography. The latter covers the general field of bacteriology, with the exception of plant diseases, this division being reserved for the second volume. The publication is well illustrated, many of the cuts showing the effect of bacteria upon plant tissue. The index covers the entire volume and will be especially useful in connection with the bibliography, which is arranged by subjects in the body of the volume.

Among the many original things in this suggestive work, the discussion of "keeping of records" and "nomenclature and classification" are of special interest. Any one who has attempted to keep an accurate history of the behavior of a plant parasite in the laboratory and in its host when the work has extended over a number of years, has felt the need of an improved system. The subject of bacterial classification has been fairly quiescent for some years, and we have been busy trying to fit the forms as they are found into MIGULA'S somewhat artificial framework. The proposition of the author to replace MIGULA'S *Pseudomonas* by *Bacterium* and send the group represented by *B. anthracis* masquerading under the name of *Aplanobacter* will come to some as a discouragement.

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[1] SMITH, E. F., Bacteria in relation to plant diseases. Vol. I. 4to. pp. xii+285. Washington: Carnegie Institution, 1905.

In spite of some minor things which seem inseparable from originality, this work is of the first quality and should be in the hands of every plant pathologist. —H. A. HARDING.

MINOR NOTICES.

Grasses of Iowa.— As a supplementary report for 1803 the Iowa Geological Survey issues part II of the *Grasses of Iowa*,[2] prepared by PAMMEL, BALL, SCRIBNER, and others. This is a descriptive and geographical study of the grasses of the state, their general and economic aspects having been treated in part I. Under each genus there is the generic description, with synonymy, a key to the species, a description of the species, often a figure, a list of localities and a map showing the distribution of each form in the state, and a statement of distribution in North America and elsewhere. There is a chapter on physiography and geology, with a map, a section on ecology, and a partial bibliography of works on grasses. The work seems very complete and should be especially serviceable to Iowa botanists. It is a pity state printers are so seldom skilful book-makers.—C. R. B.

Connecticut fungi.— The recently established natural history survey of Connecticut has begun to show results, in the publication of two bulletins listing the Hymeniales and Ustilagineae of the state. The former[3] lists 375 species in 65 genera, gives analytic keys to the genera, and illustrates the commoner species by admirable half-tones, most of which are original. The species of smuts[4] are described with lists of hosts and distribution, and notes on economic features.— C. R. B.

NOTES FOR STUDENTS.

Photosynthesis and temperature.—The interesting results of Miss MATTHAEI on temperature as a limiting factor for photosynthesis[5] have now been extended by her work in cooperation with BLACKMAN.[6] They have endeavored to interpret the quantitative variations of photosynthesis, under approximately natural conditions, in terms of the three limiting factors thereto, viz. (1) intensity of illumination, (2) temperature of leaf, (3) pressure of CO_2. When a leaf is

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[2] PAMMEL, L. H., BALL, C. R., and SCRIBNER, F. L. The grasses of Iowa. Part II, Iowa Geological Survey, supplementary report. 1903. 8vo. pp. xiv + 436, *figs. 270*. Des Moines, Iowa. 1904.

[3] WHITE, E. A., A preliminary report on the Hymeniales of Connecticut. State Geol. and N. H. Survey Bulletin 3. 8vo. pp. 81. *pls. 40*. 1905.

[4] CLINTON, G. P., The Ustilagineae or smuts of Connecticut. Idem, Bull. 5. 8vo, *pp. 45. figs. 55*. 1905.

[5] See BOT. GAZETTE **38** : 476. 1904.

[6] BLACKMAN, F. F., and MATTHAEI, G. L. C., Experimental researches in vegetable assimilation and respiration. IV. A quantitative study of carbon dioxide assimilation and leaf temperature in natural illumination. Proc. Roy. Soc. London B. **76**: 402–460. 1905.

exposed to diffuse daylight alone the amount of photosynthesis is a measure of the light, and it varies with varying light only when the amount of carbon dioxid in the atmosphere is artificially increased and the temperature is kept high. If not, photosynthesis is limited thereby and is constant though the light vary. Isolated leaves may rise more than 10° C. above a bright mercury thermometer in the sun, a result quite at variance with BROWN and ESCOMBE'S results,[7] which, however, were calculated, not observed. Further study of this point is needed.

At normal temperature leaves are not able to utilize the full amount of energy absorbed; helianthus could reach its maximum at 29° C. with about 68 per cent. full sunlight and cherry laurel with about 36 per cent. When light is the limiting factor equal intensities produce equal photosynthesis with leaves of most various structure and type. At low temperatures leaves as different as helianthus and cherry laurel have similar photosynthetic maxima, but at high temperatures these diverge. Thus at 29.5° C. the former can fix twice as much CO_2 as the latter, requiring twice as much energy to do it, of course. The essential difference in the photosynthetic activity in different leaves lies, then, in that they have different coefficients of acceleration of this function with increasing temperature. So in nature it appears that the low pressure of CO_2 (entailing slow diffusion after solution at the surfaces of the leaf cells) and the low temperatures are the serious impediments to food making.—C. R. B.

Root tubercle cultures.—Much interest has been excited during very recent years by work done in the Department of Agriculture concerning soil inoculation with various root tubercle bacteria. Widespread and rather unfortunate notoriety has been given to the work by numerous popular magazine and newspaper articles, and the impression has been gained by the public that nearly all knowledge concerning the root tubercle is to be attributed to the recent investigations conducted in the Department. This popular impression is of course erroneous. The two distinctive contributions to this subject claimed by the workers in the Department of Agriculture were that the nitrogen-gathering ability of the bacteria was heightened by new cultural methods, and that a method of transportation in dried condition, upon cotton, had been devised, whereby pure cultures could be distributed readily to farmers.

Much skepticism has existed concerning the possibility of practically heightening the nitrogen-gathering power of the bacteria, and in a recent bulletin[8] HARDING and PRUCHA claim to have demonstrated by an examination of eighteen of these cotton cultures that such packages are worthless for practical purposes since the organisms are unable to survive upon the cotton or survive in such small numbers as to be practically valueless. "Substantially identical results upon six of these packages were obtained in five separate laboratories," and the reviewer may add that similar results were obtained in his own

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[7] See BOT. GAZETTE **40**: 473. 1905.

[8] HARDING, H. A., and PRUCHA, N. J., The quality of commercial cultures for legumes. N. Y. Agr. Exp. Sta. Bull. **270**:345–385. 1905.

laboratory. The inability of the cultures to live is attributed to the method of preparation and not to any knavery upon the part of the commercial producers. A test conducted by the authors of this bulletin demonstrated the inability of the organism to survive to a satisfactory degree upon the cotton. Any intention of opposing the idea of treating the seed of legumes with living bacteria is distinctly disavowed.

It is exceedingly unfortunate that this method should have been given such wide publicity and launched as a commercial enterprise until the question as to its efficiency had been thoroughly tested.—F. L. STEVENS.

Streaming of protoplasm in mucors.—This phenomenon, although very striking and easily observed, has been little studied. The movement was noticed by WORONIN in 1866 in *Ascophanus pulcherrimus*. It was described with considerable detail in a number of species belonging to different genera by SCHRÖTER, writer of the latest account,[9] in 1897, and the conclusion was drawn that the movement was dependent upon osmotic conditions. A careful study was also made by CHARLOTTE TERNETZ in 1900, using *Ascophanus carneus*, and the conclusion was reached that it was due to the local entrance or loss of water. SCHRÖTER has confirmed and somewhat extended the work of his predecessors. For his studies he used *Mucor stol.* (as he invariably writes *M. stolonifer*) and *Phycomyces nitens*. He had better apparatus than his predecessors and was able to arrive at some conclusions with much certainty. He found that the movement was affected only very slightly by variation in the intensity of light. The action of ether, extremes of temperature, pressure, wounds, variation in amount of carbon dioxid, was similar to that of the same agents when applied to the higher plants. The streaming is found to be due to osmotic action and transpiration and therefore does not occur in a homogeneous substratum, as for instance when the fungus is wholly submerged, or in a saturated atmosphere. The streaming is not a rotation or circulation, as in the hairs of roots and stamens and in the cells of Chara, Nitella, Vallisneria, etc., but a backward and forward-movement, in which the protoplasm, vacuoles, and nuclei participate. Occasionally the acropetal movement is somewhat balanced by a thin peripheral layer of protoplasm without vacuoles setting up a basipetal movement. Usually the movement is toward one end of the hyphae for a longer or shorter time, then stops and starts again in the opposite direction.—J. C. ARTHUR.

Germination and radium emanations.—KÖRNICKE[10] has continued his study of the effect of radium emanations on the germination of ungerminated seeds which have been exposed in both the dry and wet condition. His earlier tests were made with radium bromid contained in glass tubes. In his later study he has used a much more powerful mixture which was contained in tubes having one side of

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9 SCHRÖTER, ALFRED, Ueber Protoplasmaströmung bei Mucorineen. Flora **95**: 1–30. 1905.

10 KÖRNICKE, M., Weitere Untersuchungen über die Wirkung von Röntgen- und Radiumstrahlen auf die Pflanzen. Ber. Deutsch. Bot. Gesells. **23**: 324–332. 1905.

aluminium, through which the emanations pass more readily. In all the trials he finds that although the germination is not prevented there is a period of retarded growth in the seedling. The elongation of the root or stem may be temporary or permanent according to the duration of the exposure. In the latter case the injured organ persists indefinitely without disorganization, but further growth of the seedling occurs in the form of secondary members. In the case of *Vicia Faba* such a condition will follow an exposure of only one hour; yet an exposure of fourteen days does not prevent germination. Since the retardation of growth occurs sooner in the root than in the stem of a given seedling, the author favors the explanation offered by other investigators, who have worked on entirely different material, that organs engaged in photosynthesis are more resistant to the emanations. The author's experiments offer no conclusive evidence on this point. Organs of seedlings from seeds exposed to emanations retain geotropic sensibility as long as they are capable of growth, the two capacities being concurrent. The same is true of heliotropic sensibility. His earlier view that radium emits enough luminosity to induce heliotropism, which was questioned by MOLISCH, is maintained. Important as these results are, it seems to the reviewer that their value would be much greater if obtained under standardized conditions.—RAYMOND H. POND.

Anatomy of Matonia.—TANSLEY and LULHAM describe the development and mature anatomical structure of a number of specimens of *Matonia pectinata* gathered by one of them on Mount Ophir in the Malay Peninsula.[11] The cotyledons in this species are bilobed as in the polypodiaceous ferns. Below the first leaf the central cylinder of the young stem consists of a rod of xylem, surrounded by parenchyma alone; later phloem appears on the outside of the stele and in the center as well. Subsequently the endodermis and "ground tissue" likewise appear within the stele, which becomes typically siphonostelic. By a process of "local dilatation of the m rgin of the leaf gaps" an internal mass of fibrovascular tissue appears, which ultimately becomes tubular and lies within the original fibrovascular tube. This inner tubular fibrovascular bundle subsequently gives off an internal tracheary strand, which may also become tubular, so that there may be in Matonia as many as three tubular bundles lying one within the other. These join each other only in the region of the nodes. The authors consider the internal fibrovascular system as a storage tissue only, since it has no direct connection with the roots, which are attached to the external cylinder, as in other ferns of this type. The views as to the morphological nature of the complex fibrovascular system of the stem in this species may be regarded as "orthodox," since the conclusion is reached that it constitutes a single stele. The hypothesis that the pith is intruded cortex is accordingly rejected, since the authors are of the opinion that the only trustworthy criterion as to the morphological value of tissues is to be derived from a study of their relation to the primary meristems of the growing point.—E. C. JEFFREY.

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[11] TANSLEY, A. G., and LULHAM, Miss R. B. J., A study of the vascular system of *Matonia pectinata*. Annals of Botany **19**:476–519. *pls. 31–33*. 1905.

Chloroplasts of sun and shade plants.—LUBIMENKO[12] refuses to accept as a general law the statement formulated by TIMIRIAZEFF that the maximum photosynthesis occurs under an intensity of illumination equal to about one half that of direct insolation. By measuring the rate of photosynthesis of ombrophilous plants (Tilia and Abies) and of ombrophobous plants (Betula and Pinus) under both artificial and natural light he finds that plants differ as to the minimum insolation necessary to initiate photosynthesis. In this result the author finds basis for the conception of a specific quality of the chloroplasts. Further investigation convinces him that the curve of photochemical work is determined primarily by the specific quality of the chloroplasts and by the anatomical structure of the leaf. The influence of the latter factor is particularly evident during periods of moderate sunshine, but the potency of the former is manifest under insolation of high or low intensity.

The chloroplasts of the ombrophilous plants have greater dimensions and a sensibility almost five times greater than that of the chloroplasts of ombrophobous plants. Other tests also indicate that the pigment is more concentrated in the chloroplasts of the former.

While auxiliary data support the author's main conclusion, the chance of error through imperfect technique and ignorance of all the factors is so great that final conclusions are better withheld.—RAYMOND H. POND.

Microsporangia of Sphenopteris.—It has been suggested that the microsporangia of *Lyginodendron Oldhamium*=*Sphenopteris* (*Crossotheca*) *Höninghausii* might be found on *Telangium Scotti*. KIDSTON[13] concludes from its structure that *Telangium Scotti* cannot be the microsporangia of *Sphenopteris Höninghausii*. In no instance was organic connection between the two demonstrated.

He found a few microsporangiate pinnae referable to *Crossotheca* Zeiller in organic connection with barren pinnae of *Sphenopteris Höninghausii*. The fertile pinnule is oval, entire, and attached to the rachis by a short pedicel, which is thickened very slightly upwards before merging into the pinnule, to which it seems to be united for a short distance. The pinnules appear to be rather thick, and the vascular bundle which enters the pinnule divides into two branches, which separate slightly from each other. Each fertile lobe bears six to eight broadly lanceolate, sharply pointed, bilocular microsporangia, which in early stages bend inward, forming a small hemispherical bunch, with their apices meeting in the center, Later, the microsporangia spread outward and appear as a fringe hanging from the margin of the pinnule. The microspores are either slightly oval or circular and measure 50 to 75 μ in diameter. The walls are roughened, being covered with minute blunt points. The tri-radial ridge, marking the line of cleavage of the tetrads, is sometimes apparent.—W. J. G. LAND.

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[12]LUBIMENKO, M. W., Sur la sensibilité de l'appareil chlorophyllien des plantes ombrophiles et ombrophobes. Rev. Gén. Bot. **17**:381–415. *pls.* 10, 11. 1905.

[13]KIDSTON, R., Preliminary note on the occurrence of microsporangia in organic connection with the foliage of Lyginodendron. Proc. Roy. Soc. B **76**: 358–360, *pl. 6*. 1905.

Photic sense organs.—GUTTENBERG[14] has demonstrated that two of the ombrophilous species of his local flora have a photo-sensitive epithelium, whose response consists in maintaining the leaf in the transverse heliotropic position. The mechanism is essentially the same as was found by HABERLANDT in the so-called velvet leaves, so abundant among the ombrophilous species of the tropical hydrophytic forests. The epidermal cells function as converging lenses, so that the protoplasmic membrane which covers the floor of the cell is not uniformly illuminated. In HABERLANDT'S studies the bright spot was centrally located, but GUTTENBERG finds that for his species that it is excentric, because the papillosity is not centrally located. The result is the same in both cases, for the leaf is attuned to the distribution of interior illumination which exists when the leaf is in the transverse position. Actual tests showed that the petiole is not a factor in securing this position. Curiously enough the leaf assumes the horizontal position in diffuse light, such as occurs under the open sky on a cloudy day. In this case, however, the internal distribution of light is the reverse of that which exists under parallel rays, the central area of the floor wall being dark with a margin of increasing brightness. The stimulus apparently consists in an unequal illumination of the cell lumen.—RAYMOND H. POND.

Nature of chromatophores.—MERESCHKOWSKY[15] holds that these bodies are not organs of the plant cell and never have been, but are foreign organisms which penetrated into the colorless plasma of the cells and live there as symbionts. In support of this notion he adduces the facts that the chromatophores multiply continuously by division and do not arise *de novo;* that they are in high degree independent of the nucleus; that they are completely analogous with zoochlorellae and zooxanthellae which inhabit hydras, infusoria, etc.; that there are organisms, (e. g., the lower Cyanophyceae, such as Aphanocapsis and Microcystis) which can be considered as free-living chromatophores; that certain Cyanophyceae actually live as symbionts in the cell plasma. This theory he thinks, is the only possible explanation of the polyphyletic origin of primeval plants, which were merely amoebae and flagellates into which Cyanophyceae migrated; that the green, red, and brown Cyanophyceae account for the algae of these colors; that the plant cell-wall is due to the formation, by the symbiotic chloroplasts, of carbohydrates easily polymerized into cellulose ; which wall makes impossible the further taking of solid food and entails the quiescent nature and simple organization of plants, minus nerve, muscle, and psychic life. Here is another pyramid of theory resting on its apex.—C. R. B.

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[14]GUTTENBERG, H. R., VON, Die Lichtsinnesorgane der Laubblätter von *Adoxa Moschatellina* und *Cynocrambe prostrata*. Ber. Deutsch. Bot. Gesells. **23**:265–273. *pls. 10, 11.* 1905.

[15] MERESCHKOWSKY, C., Ueber Natur und Ursprung der Chromatophoren im Pflanzenreiche. Biol. Centralbl. **25**: 593–604. 1905.

Idioblasts of Cruciferae.—SCHWEIDLER[16] has decided to assign a systematic value to the peculiar idioblasts of the Cruciferae. The author at present reserves judgment as to their generic value, though this is expected to be established by further work. He has no doubt, however, that suborders and tribes can be accurately defined. On this basis he divides the family into three suborders. The first is characterized by the presence of idioblasts which contain chlorophyll and which are located exclusively in the mesophyll. The idioblasts of the second suborder occur in the vascular tissue and differ from those of the first group in not containing chlorophyll. The third suborder is composed of members which have both kinds of idioblasts. Just what would happen to the systematic standing of an individual so unfortunate as to have had the development of its idioblasts inhibited is certainly not for the reviewer to say, but in view of the urgent necessity of establishing systematic work upon an experimental basis rather than morphological, it is difficult to escape the conviction that more or less futility is involved in all those efforts of which this paper is an example.—RAYMOND H. POND.

Araucarineae.—A preliminary note by THOMSON[17] states that in Agathis there are many supernumerary nuclei in the pollen tube and that in Araucaria as many as thirty were counted. The pollen tube grows along the surface of the ligule for 22^{cm} or more before entering the micropyle. The anatomy of the ovule and development of the archegonia, as well as of the pollen tubes and megaspore membranes indicate that the Araucarineae occupy a very isolated position among the Coniferales.

SEWARD and FORD in an abstract of a paper[18] read before the Royal Society Dec. 14, 1905, indicate the scope of an extensive investigation of the Araucarieae. The section headings are: Introduction, distribution, diagnosis and synonymy, seedlings, root and stem anatomy, leaves and leaf traces, reproductive shoots, fossils, and phylogenetic considerations and conclusions.

The most important conclusion is that the group, unlike the Cycadales, has been derived from lycopodiaceous ancestors. The Araucarieae differ so greatly from the other Coniferales that the authors suggest the substitution of the term, *Araucariales* for *Araucarieae*.—CHARLES J. CHAMBERLAIN.

Inhibitory action.—ERRERA[19] suggests that the non-development of lateral branches or their growth in a particular position (e. g., of certain conifers) is determined by inhibitory stimuli (de nature catalysatrice si l'on veut) traversing either bark (Araucaria) or all living cells (Picea). We may conceive, he says, the apex of the stem or root as a sort of tyrant who forbids the subjacent

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[16]SCHWEIDLER, J. H., Die systematische Bedeutung der Eiweiss- oder Myrosinzellen der Cruciferen nebst Beiträgen zu ihrer anatomisch-physiologischen Kenntniss. Ber. Deutsch. Bot. Gesells. **23**:274–285. *pl.* 1905.

[17]THOMSON, R. B., Preliminary note on the Araucarineae. Science **22**:88. 1905.

[18]SEWARD, A. C., and FORD, Sibille, O., The Araucarieae, recent and extinct.

[19]ERRERA, L., Conflicts de préséance et excitations inhibitoires chez les végétaux. Mém. Soc. Roy. Bot. Belgique **42** : 27–43. 3. Aug. 1905.

branches to erect themselves or in other cases to develop, though they have the same tendency to do so as he; their geotropism or their power of growth is held in check by his own. Suppress the apex, let it die or become enfeebled, and the subjugated branches lift their heads. Several could erect themselves and take the lead, and that is sometimes observed. But ordinarily a new conflict for precedence occurs among the branches; the one nearest the apex or the most vigorous near one early asserts its supremacy and in its turn keeps its rivals at its feet. *Cf.* the independent and almost simultaneous proposal of the like idea by McCallum, Bot. Gazette **40**: 262. Oct. 1905.—C. R. B.

Ecological survey.—Pethybridge and Praeger[20] have added another vegetation map and ecological description to the list of vegetation surveys of the British Isles. The area discussed lies south and west of Dublin. After a historical introduction the geology, physiography, floristics, and survey methods are briefly explained. The vegetation is divided primarily into littoral, agrarian, hill-pasture, and moorland zones, and the woodlands. The zones are further subdivided into associations. These are described in detail and as far as possible related to the factors determining their occurrence. The text is accompanied by a map and five excellent plates of vegetation types. The paper will prove of especial interest to those who have followed the work of R. Smith, W. Smith, and Lewis in Scotland and England.—E. N. Transeau.

Alternation of generations in animals.—In criticism of Chamberlain's paper on this subject[21] Lyon[22] holds that the phylogeny of animal gametes gives no evidence of their being reduced or vestigial generations, comparable with the gametophytic generation in plants; similarity of cytological processes does not prove identity of morphological value in the two cases. He refers to the alternation in Hydrozoa, and calls attention to the earlier proposal by Beard and Murray of a theory similar to Chamberlain's. In reply Chamberlain maintains[23] that his critic fails to distinguish between a gametophytic generation and a gametophytic plant. He holds that the generations in Hydrozoa do not alternate in the botanical sense, and points out that although reduction of the gamete-bearing generation has not been proved for animals, there is strong evidence for its having occurred in plants.—M. A. Chrysler.

Mechanics of secretion.—Pantanelli[24] has attempted to ascertain whether or not true secretion of enzymes occurs. He defines secretion as "the emission

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[20]Pethybridge, G. H. and Praeger, R. L., The vegetation of the district lying south of Dublin. Proc. Roy. Irish Acad. B. **25**: 124–180. 1905.

[21]Bot. Gazette **39**: 137–144. 1905.

[22]Lyon, H. L., Alternation of generations in animals. Science N. S. **21**: 666–667. 1905.

[23]Chamberlain, C. J. Alternation of generations in animals. Science N. S. **22**: 208–211. 1905.

[24]Pantanelli, E., Meccanismo di secrezione degli enzimi. Annali di Bot. **3**: 113–142. 1905.

of substances by living protoplasm, a thing possible through a self-regulated change in the condition of permeability of the plasmatic membranes such that the organism is able at pleasure to reverse it." He finds that the ferment of Roman bread and Chianti wine truly secretes invertase, by the augmentation of the permeability of the protoplasm during the period of fermentative activity. This increased permeability is general, various salts escaping more freely at the same time. In *Mucor stolonifer*, however, the emission of invertase seems to have the character of a free exit of materials from dying parts, coincident with spore formation. Whether it has a true but weak secreting power remains for further study.—C. R. B.

Respiration.— PALLADIN distinguishes[25] three sources of the respiratory CO_2 of plants: (1) nucleo-CO_2 produced by the action of enzymes, which, partly soluble, partly insoluble in expressed sap, are intimately bound up with the protoplasm; (2) stimulation-CO_2, formed by the protoplasm itself (apparently directly) under the action of stimuli; (3) oxydase-CO_2, produced by various oxidases. The process which characterizes animal and plant life consists in the excretion of nucleo-CO_2 which is formed by decomposition without the participation of atmospheric oxygen. Intramolecular respiration is a primary phenomenon, whose CO_2 is principally nucleo-CO_2 and in some cases also stimulation-CO_2. But alcoholic fermentation is no simple phenomenon, and, as KOSTYTSCHEW has shown, must be distinguished from intramolecular respiration.— C. R. B.

Apple and pear rot.— LONGYEAR[26] has published the results of his study of a rot of apples and pears due to an undescribed species of Alternaria. The same disease has so far been found in California, Colorado, and Michigan, and in Colorado is one of the most widely distributed and common diseases of apples. PADDOCK[27] was the first to call attention to it. In the case of the apple the disease attacks the fruit only, but it attacks the fruit, leaves, and young sprouts of the pear. In the apple it appears frequently first at the blossom end of the fruit and, in the case of sorts having a deep calyx-tube, a core-rot may occur. The disease may be controlled by spraying with Bordeaux mixture and plowing under or removing the diseased fruits in which the fungus is able to pass the winter.— E. MEAD WILCOX.

Reduction division.—The earliest organisms were doubtless non-sexual. In phylogeny, according to SCHAFFNER,[28] the conjugation of nucleated cells introduced

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[25] PALLADIN, W., Ueber den verschiedenen Ursprung der während der Atmung der Pflanzen ausgeschiedenen Kohlensäure. Vorlaufige Mitteilung. Ber. Deutsch. Bot. Gesells. **20**: 240–247. 1905.

[26] LONGYEAR, B. O., A new apple rot. Bull. Col. Agric. Exp. Stat. 105: 1–12. *pls. 1–4.* 1905.

[27] PADDOCK, W., A new apple disease. Rept. Col. Exp. Stat. **17**: 99. 1904.

[28] SCHAFFNER, JOHN H., The nature of the reduction division and related phenomena. Ohio Naturalist **5**: 331–340. 1905.

a disturbance into the life cycle and a reduction division of some kind became an inevitable accompaniment. The places at which a reduction division might, theoretically, become established in the life cycle are presented in diagram and described. A comparison between the life cycles of plants and animals is also illustrated by a diagram. SCHAFFNER believes that in the higher animals the condition appears to be similar to that found in Fucus.

The significance of a transverse division of chromosomes in interpreting the phenomena of MENDEL'S law is illustrated and discussed.—CHARLES J. CHAMBERLAIN.

Migration of salts.— In an extensive investigation of the content of nitrogen, phosphoric acid, sodium, and potassium in cultivated plants, both field and pot grown, at different periods of their development, it has been found[29] that in different plants the maximum absorption is completed at different periods, barley, spring wheat, peas, and mustard attaining this maximum at flowering, while potatoes reach it at maturity. These substances do not remain at a maximum, but in the plants other than potatoes and with the exception of phosphoric acid, migrate back, in great part, to the soil; this seems to depend on the amount of a given substance available, being greater when, say, potassium is lacking than if the appropriate materials are all supplied.—C. R. B.

Anatomy and affinity.—Another observer, SARTON, has attempted to ascertain how much help is to be had from histology in determining the validity of Jordanian species as contrasted with Linnean.[30] He studied allied plants, submitted them to cultivation under diverse conditions and then examined their structure. In some cases there were constant anatomical characters distinguishing apparently closely allied forms. On the other hand the characters were as often elusive and evidently directly adaptive. Plants long cultivated in the Jardin des Plantes and at Fontainebleau showed no anatomical differences from wild ones of the same species. Nor were there differences between the varieties having different colored flowers.—C. R. B.

Scotch moors.—The succession of plants in the moors of the Scottish southern uplands has been studied by LEWIS.[31] He finds that in all the localities visited the peat "shows a definite stratification of plant remains, indicating a swing from woodland to heath and moss, and again to woodland. In some districts, an arctic plant-bed is interposed between the lower and upper woodland beds." The vegetation changes are probably correlated with climatic changes at the

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[29] WILFARTH, H., RÖMER, H., and WIMMER, G., Ueber die Nährstoffaufnahme der Pflanzen in verschiedenen Zeiten ihres Wachstums. Landw. Versuchsstat. **63**: 1–70. *pls. 3.* 1905.

[30] SARTON, A., Recherches expérimentales sur l'anatomie des plantes affines. Ann. Sci. Nat. Bot. IX. **2** : 1–115. *pls. 14.* 1905.

[31] LEWIS, F. J., The plant remains in the Scottish peat mosses. Pt. I. The Scottish southern uplands. Trans. Roy. Soc. Edin. **41**[3]:699–722. 1905.

close of the glacial period. He concludes also that the differences in the basal deposits of these moors as compared with those of the higher Cross Fell district (upon which he reported earlier) indicate the relative time of origin.—E. N. TRANSEAU.

Aberrant chromosomes.—The discovery of chromosomes of different sizes in the same nucleus in plants suggests that the attention of botanists be called to the terminology just proposed by MONTGOMERY for aberrant chromosomes in Hemiptera.[32] The term chromosomes is retained when all the chromosomes of a nucleus are alike; when they are unlike, the name *autosoma* or *autosome* is applied to a chromosome of the usual form, and *allosoma* or *allosome* to an aberrant chromosome. Unpaired allosomes are *monosomes*, and paired allosomes are *diplosomes*.—CHARLES J. CHAMBERLAIN.

Iron-algae.—After observation in the field and a study of cultures, GAIDUKOV[33] concludes that a Conferva found by him in overflow pools of the Ocka river near Rjasan accumulates iron oxid from the waters, just as other algae do calcium carbonate or silica. He thinks such iron secretion not peculiar to the bacteria, but characteristic of many organisms, not as a necessary life-process, but as an adaptive one. In the present case it seems to be protective to the akinetes, which, weighted down by the iron oxid, sink to the bottom and so pass the winter.—C. R. B.

Photosynthesis and electricity.— POLLACCI[34] announces that electric energy, when it does not exceed a given intensity, promotes very much the formation of starch in leaves, and that this effect is greater with a continuous current passing directly into the interior of the organs. Electrified leaves almost deprived of light in some cases showed starch formation, when, in the same illumination, unelectrified leaves did not. In view of the recent English work on photosynthesis these conclusions should be received with reserve.— C. R. B.

Formation of proteids.—MONTEMARTINI[35] is attacking this much investigated problem. His first paper clears the ground, records once more a good part of the extensive bibliography, and details two experiments, which lead to the conclusion that the production of proteids is greater in light than in darkness, and greater in light and air minus CO_2 than in light and normal air. Likewise it is fivefold greater in the day than in the night, and he proposes to analyze the relation of light to this result in his later experiments.—C. R. B.

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[32]MONTGOMERY, THOS. H., The terminology of aberrant chromosomes and their behavior in certain Hemiptera. *Science* **23**:36–38. 1906.

[33]GAIDUKOV, N. , Ueber die Eisenalge Conferva und die Eisenorganismen des Süsswassers in algemeinen. Ber. Deutsch. Bot. Gesells. **23** : 250–253. 1905.

[34]POLLACCI, G., Influenza dell' electricità sull' assimilazione clorofillina. Nota preliminare. Atti Ist. Bot. Pavia II. **11**: 7–10. 1905.

[35]MONTEMARTINI, L., Primi studi sulla formazione delle sostanze albuminoidi nelle piante. Atti R. Ist. Bot. Pavia II. **10**: 1–20. 1905.

Chemotaxis of sperms of Equisetum.—LIDFORSS,[36] to avoid anticipation by SHIBATA, has made a preliminary announcement of his discovery that the spermatozoids of Equisetum are markedly chemotactic toward solutions of malic acid especially, and also to maleic acid and its salts. Only indifference is shown to solutions of fumaric acid or of its salts. The threshold concentration of malic acid he finds to be about M/10000. Aerotaxis, which had been previously observed in the case of Marchantia spermatozoids, could not be demonstrated.—RAYMOND H. POND.

Welwitschia.—*Tumboa mirabilis* is so little known that any fresh observations are welcome. PEARSON[37] succeeded in securing material showing the development of microsporangia, microspores, megasporangia and megaspores. Observations were made upon the habit, habitat, and climatic conditions. It is probable that the plant is partially, if not wholly, insect-pollinated, and that the processes of fertilization and maturation of the seed take place more rapidly than in other gymnosperms.—CHARLES J. CHAMBERLAIN.

The cycadean integument.—This is discussed in a recent paper by Miss STOPES,[38] who takes this occasion to compare the structures of the cycad ovule with those of the fossil Lagenostoma. The single integument of the living cycads is regarded as a double structure representing two integuments of some ancestral form. The plane of fusion of the two integuments has been between the inner and outer layers of the stony coat, or between the stone and the outer flesh. —CHARLES J. CHAMBERLAIN.

A rust-resistant cantaloup.— BLINN[39] finds that the Pollock strain of cantaloups is resistant to the rust or blight which is a common and serious disease in the Rocky Ford district of Colorado. This resistance he found was transmitted through seed selected from resistant plants, and hence seed selection becomes a very practical method of controlling this destructive disease wherever it may occur. The disease is due to the fungus *Macrosporium cucumerinum* E. & E.—E. MEAD WILCOX.

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[36]LIDFORSS, B., Ueber die Chemotaxis der Equisetum-Spermatozoiden. Ber. Deutsch. Bot. Gesells. **23**:314–316. 1905.

[37]PEARSON, H. H. W., Some observations on *Welwitschia mirabilis* Hooker. Abstract of a communication to the Royal Society of London, Nov. 23. 1905.

[38]STOPES, MARIE C., On the double nature of the cycadean integument. Annals of Botany **19**:561–566. 1905.

[39]BLINN, P. K., A rust-resistant cantaloup. Bull. Col. Agric. Exp. Stat. 104: 1–15. *pls. 1–10.* 1905.

NEWS.

DR. JOHN W. HARSHBERGER is delivering a course of ten lectures on North American trees before the Wagner Free Institute of Science in Philadelphia.

DR. PEHR OLSSON-SEFFER has left Leland Stanford University and has accepted a position in connection with an experiment station in Mexico, devoted to the investigation of the growing of rubber plants. His address, which correspondents are requested to note, will be La Zacualpa Botanical Station, Escuintla, Chiapas, Mexico.

THE ANNOUNCEMENT for 1906 of the Lake Laboratory, maintained by the Ohio State University at Cedar Point, on Lake Erie, has been issued. The only instructor in botany for the season is Prof. MALCOLM E. STICKNEY, assistant professor of botany, Denison University. Consequently the courses are limited to one in general botany and one in ecology. The course opens June 25th, and closes August 3d.

THE MINNESOTA SEASIDE STATION on the Straits of Fuca, Vancouver Island, opens its doors for the sixth annual session, July 8, 1906. Owing to the low rates to the Pacific coast which will be in force, this promises to be an important year in the history of the Station. Those contemplating marine study and research are invited to write to Professor Conway MacMillan, University of Minnesota, Minneapolis, for the illustrated announcement of the Vancouver Island Laboratory-Camp.

DR. ALBERT SCHNEIDER has resigned his position as professor of botany, pharmacognosy, and materia medica at the California College of Pharmacy and has been appointed pathologist and physiologist of the Spreckels Sugar Company. DR. HENRY B. CAREY, formerly assistant to DR. SCHNEIDER, has been elected to fill the vacancy created by the latter's resignation. DR. SCHNEIDER is now giving his entire time to the investigation of the so-called California sugar beet blight, which has been the cause of great losses to California beet growers.

THE ANNUAL announcement of the Marine Biological Laboratory at Woods Hole, Mass., shows that the laboratories will open on July 5th, the regular courses of instruction extending from that date to August 16th. The department of botany for this year will be manned by DR. GEORGE T. MOORE, of Washington, D. C., and DR. JAMES J. WOLFE, of Trinity College, N. C. Miss LILIAN J. MACRAE will act as collector. Correspondence regarding botanical courses should be addressed to Dr. MOORE at the Cosmos Club, Washington.

THE *Association internationale des Botanistes* has shown commendable activity not only in the conduct of the *Botanisches Centralblatt* but also in arranging for a supply of pure cultures of fungi and algæ. Now it further announces a long list

of places from which it is ready to supply material for demonstration or investigation to members of the society. The list is too long to be republished, but it is evident that one can secure working materal from a wide range of localities. Correspondence relating to such material should be addressed to the secretary, Professor J. P. LOTSY, Leyden, Holland.

BEGINNING with January 1, 1906, the form of the publications, which in the past have appeared as bulletins of the Bureau of Government Laboratories in the Philippines, will be changed to a journal to be known as the *Philippine Journal of Science*. This publication will include original articles by members of the staff of the Bureau of Science, as well as scientific papers submitted for publication by other officials of the Philippine government and by individuals not officially connected therewith. The journal will include researches in botany, zoology, chemistry (including physiological chemistry), serums and prophylaxis, mineralogy, geology, paleontology, mining, and mineral resources. The journal is to review work which is being accomplished and to present such original results as are obtained. The subscription price is $5 (U. S.) per year. It will be possible to secure reprints of any particular series of the articles at reduced prices. The journal will be edited by Dr. PAUL C. FREER, director of the Bureau of Science, with Dr. RICHARD P. STRONG, chief of the biological laboratory, and Mr. H. D. MCCASKEY, chief of the division of geology and mining, as co-editors.

IN THE SUMMER of the present year a permanent Station for the study of arctic science will be established on the south coast of Disco Island in Danish West-Greenland. The cost of the foundation is defrayed by a gift from Mr. A. HOLCK, of Copenhagen, and the Danish government has promised an annual grant of 10,000 kroner ($3000) toward its maintenance. A laboratory, equipped with appliances and instruments especially for biological researches, will be attached to the Station, and for the present two work-places will be furnished for visiting naturalists. The visitors will have the free use of the instruments, traveling outfit, and library of the Station. Lodging will be free and a small fee will be charged only for board. The first visitors can be received in 1907, and notices, inviting application, will be issued in due course. A library of arctic literature is to be founded at the Station and to be made as complete as possible, but on account of the limited resources of the Station and the vastness of the literature, only a small proportion of it can be purchased. The Director of the Station, M. P. PORSILD, asks botanists to be good enough to come to its assistance by giving to this library works on arctic and antarctic nature, and especially on arctic biology. The publications of the Station will be sent in return, and the Station will be glad to render any service in its power. Up to May 1 Director PORSILD may be addressed at Copenhagen S., Denmark.

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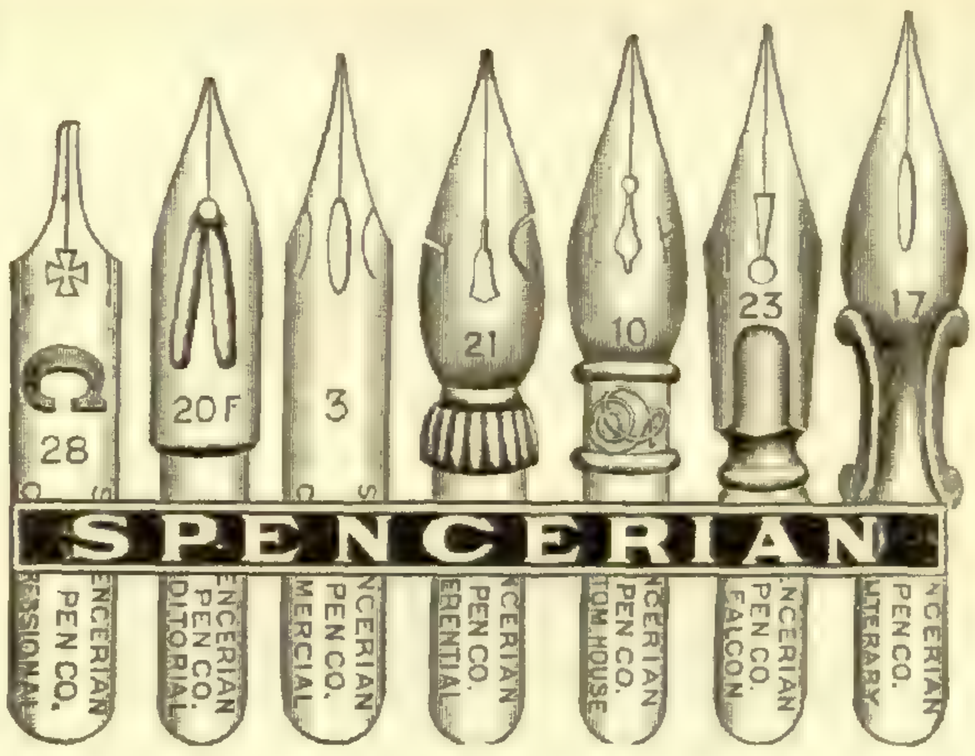

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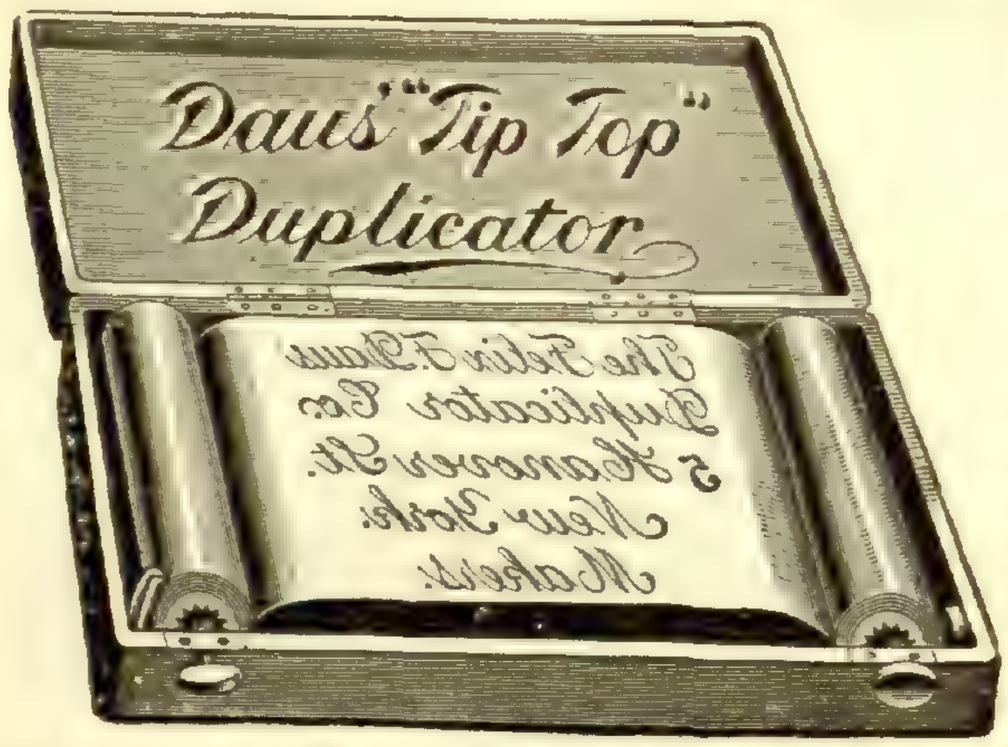

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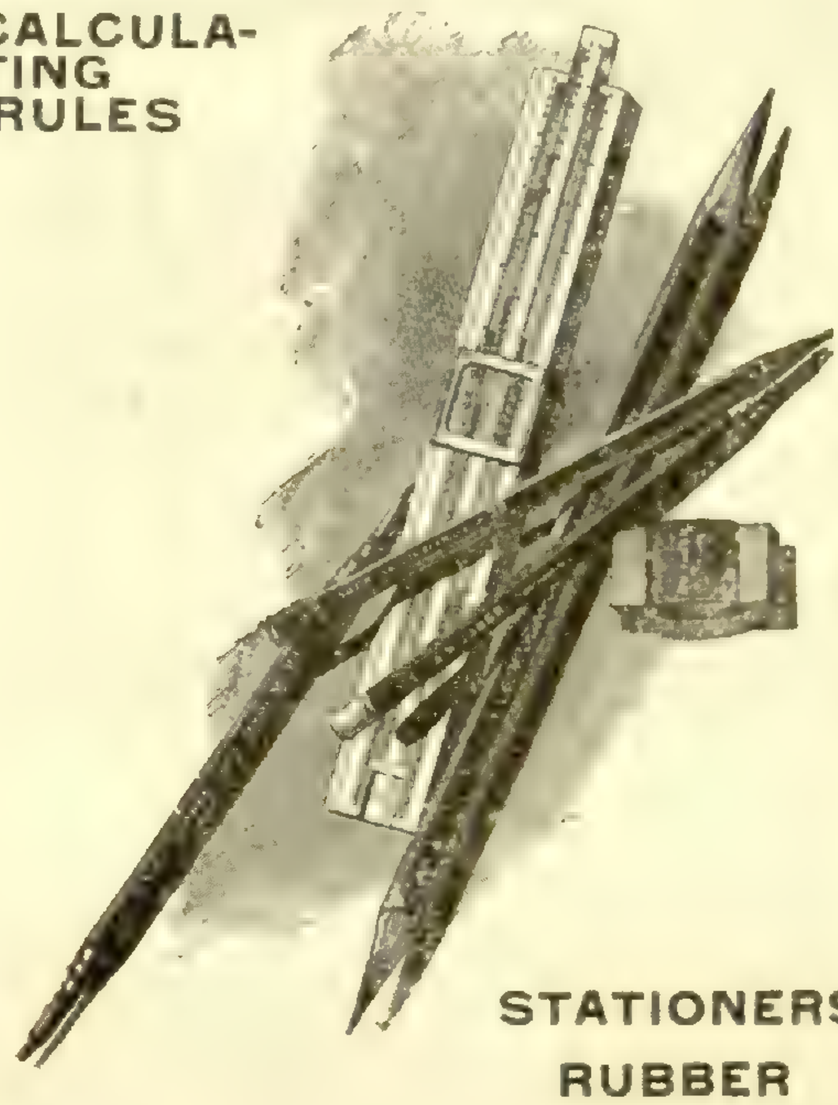

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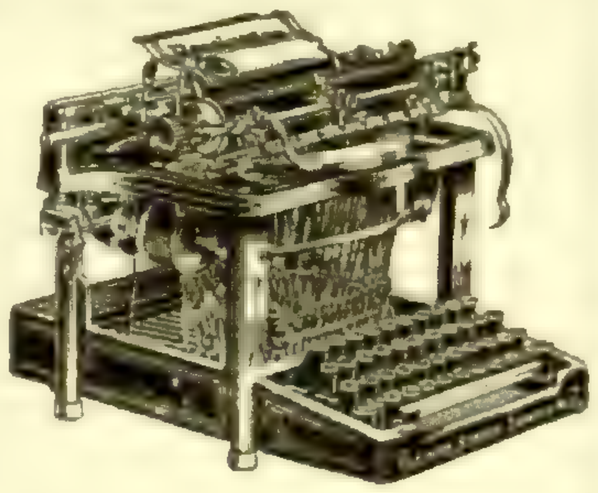

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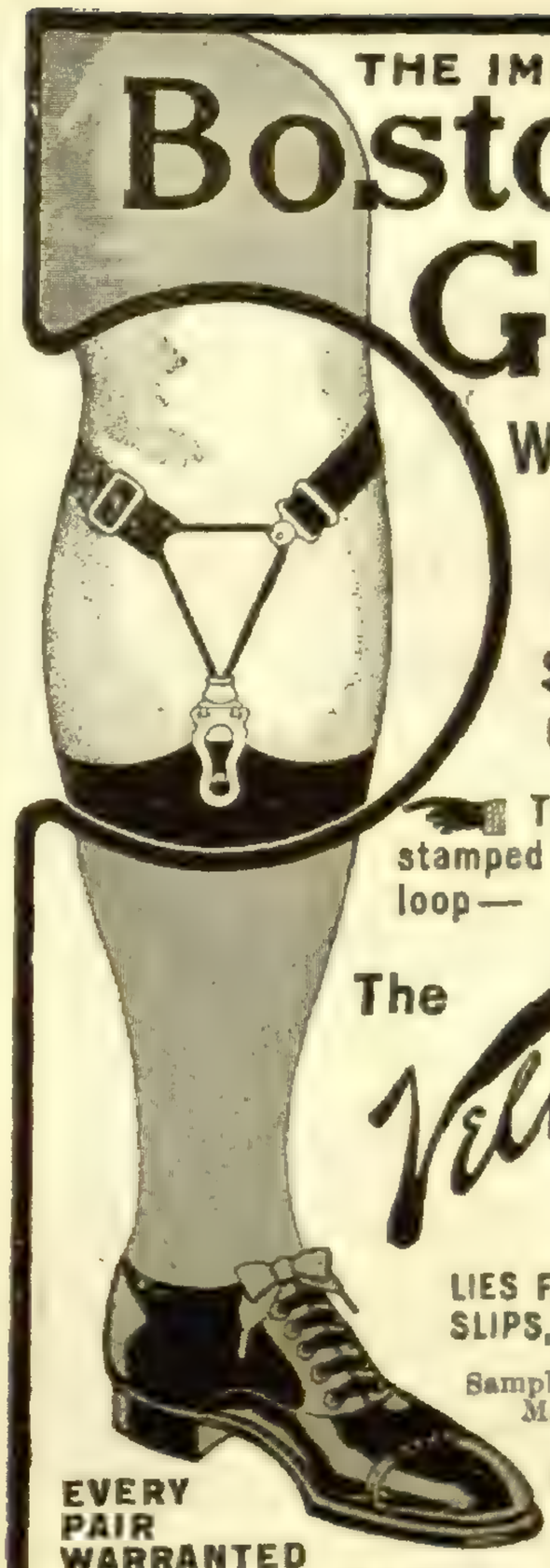

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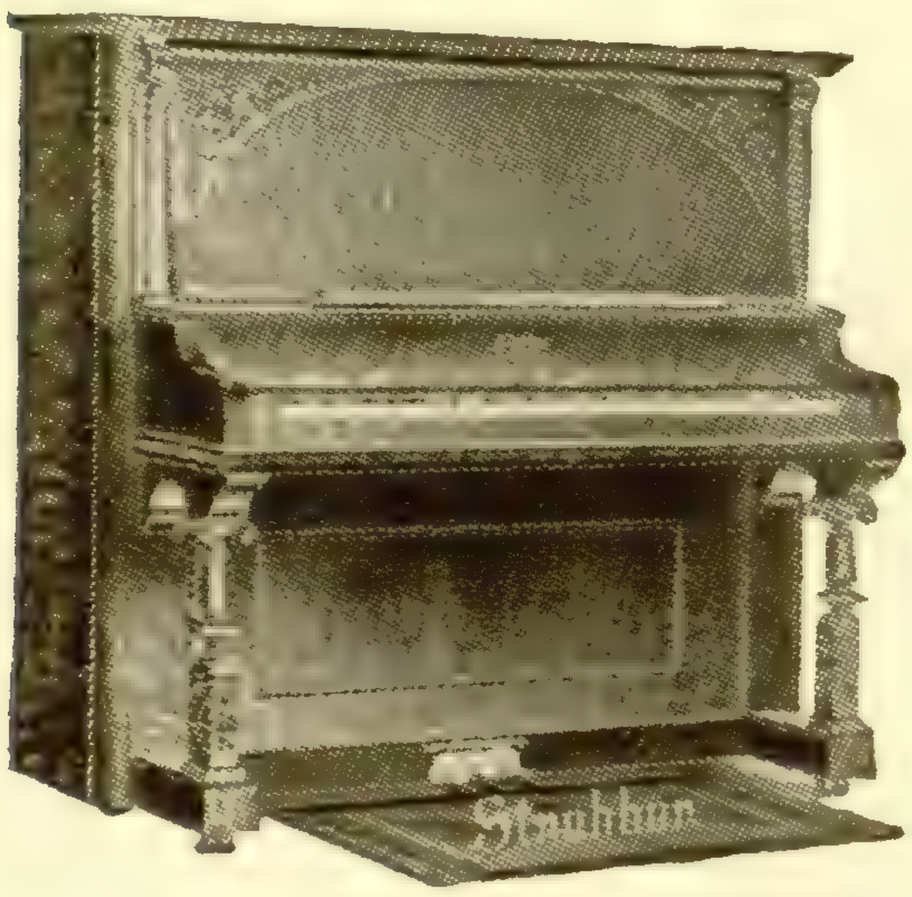

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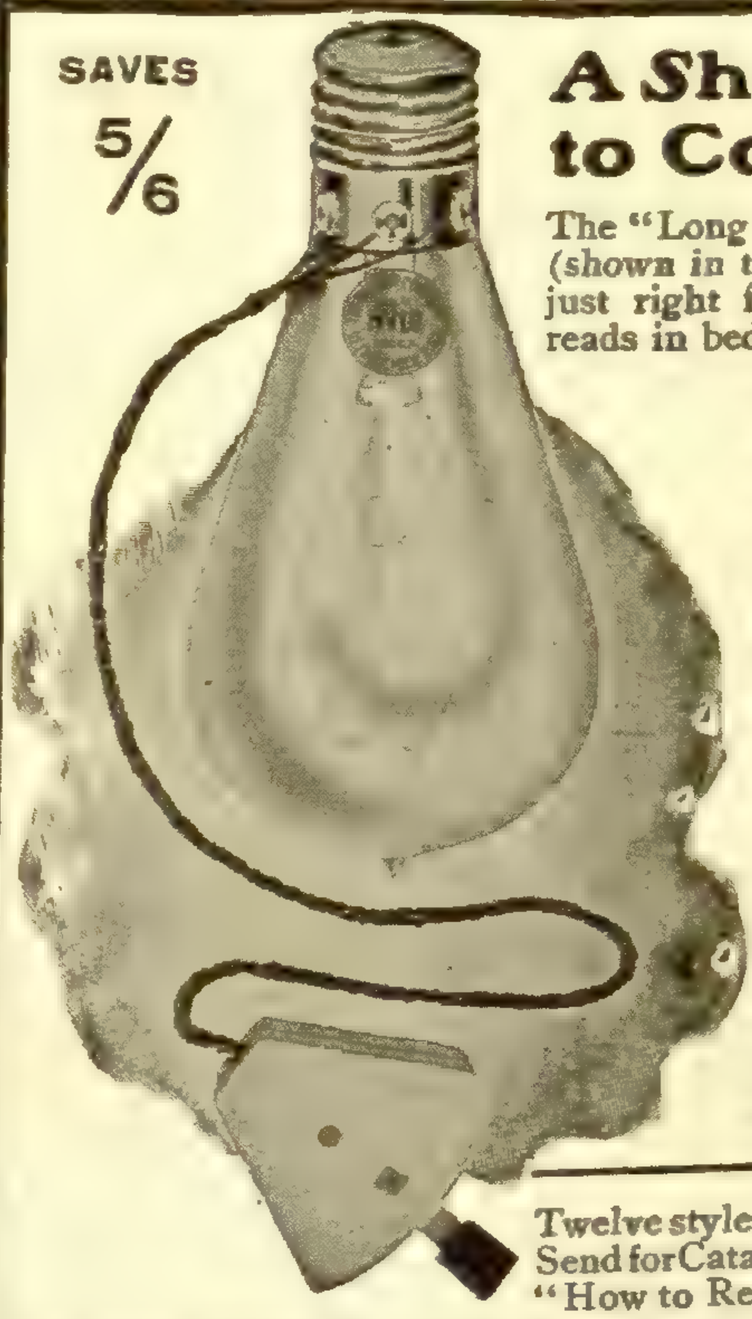

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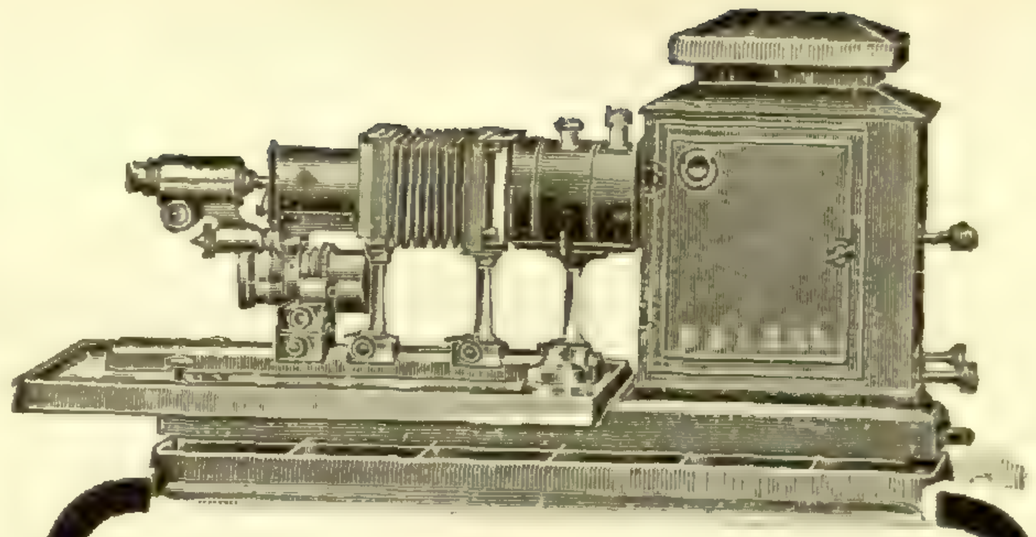

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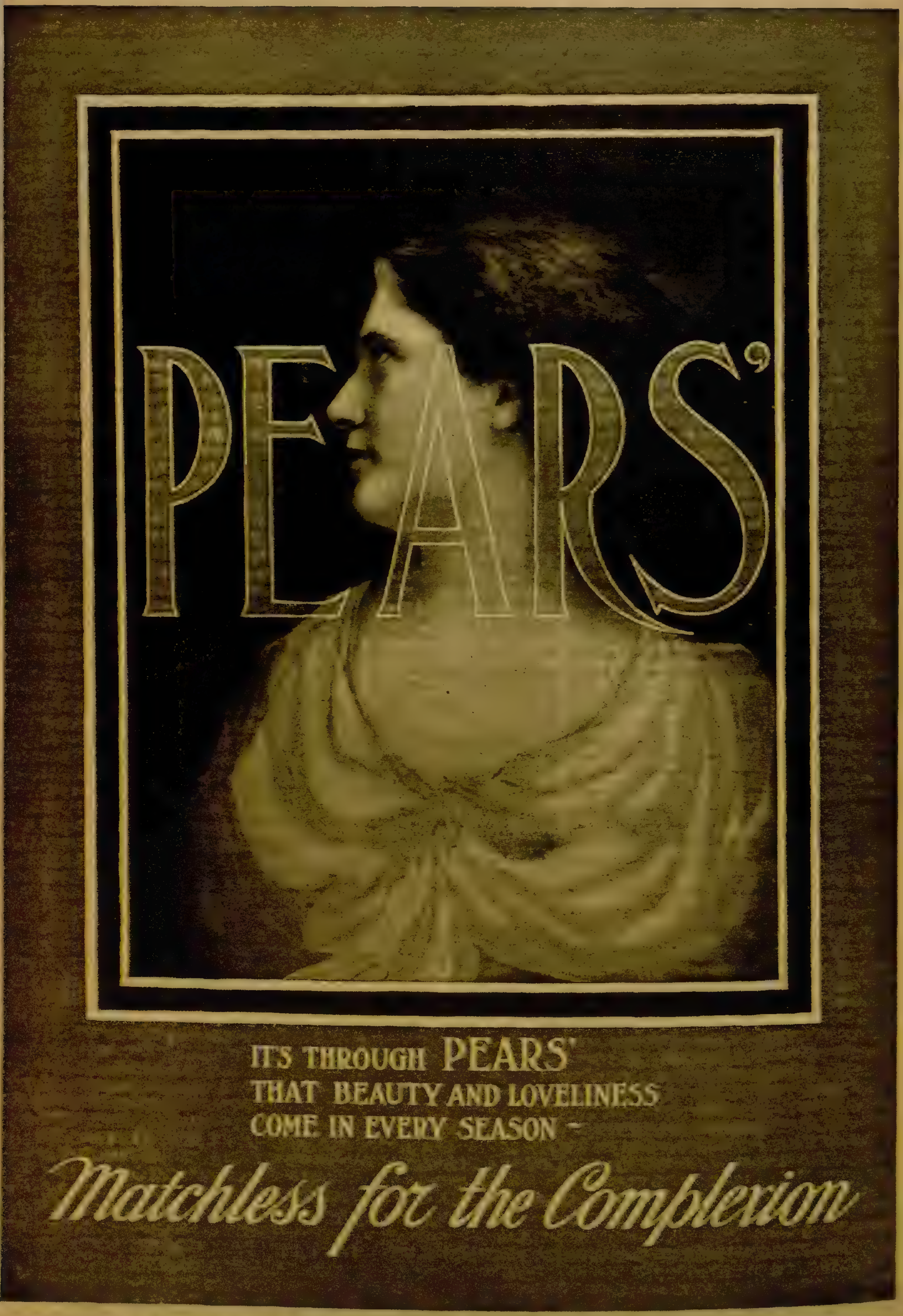

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Vol. XLI, No. 4 **Issued April 28, 1906**

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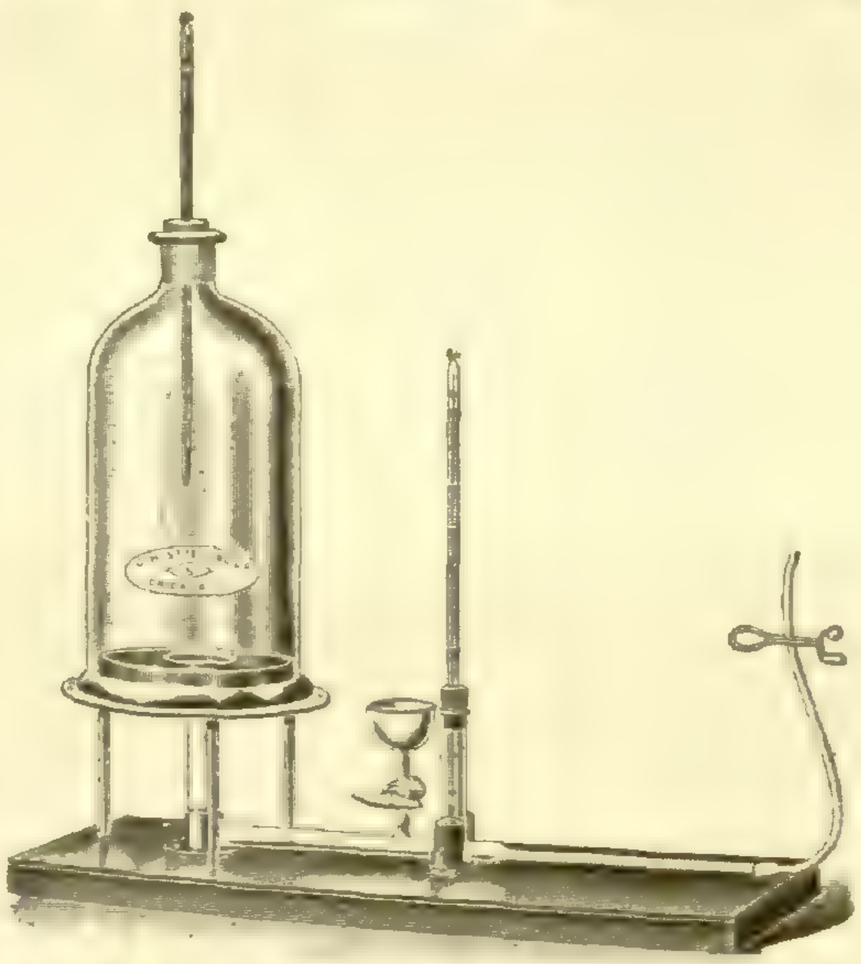

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VOLUME XLI NUMBER 4

BOTANICAL GAZETTE

APRIL, 1906

CYTOLOGICAL STUDIES ON THE ENTOMOPHTHOREAE.

II. NUCLEAR AND CELL DIVISION OF EMPUSA.

EDGAR W. OLIVE.

(WITH PLATE XVI[1])

THE division of the nuclei in Empusa has been found in the course of this investigation to resemble closely that described for Amoeba, Euglena, and other Protozoa. Such a primitive type of nucleus, which has been regarded as the typical protozoan form, has not so far been observed in the Metazoa, nor have any of the lower plants heretofore revealed a type of nucleus in which the "division-center" is permanently intranuclear. Such a type has been called by BOVERI (:**00**, p. 183) a "centronucleus," since it contains within itself a center of division which he assumes may be either in diffuse or concentrated form.

The varieties of protozoan nuclei and the types into which they may be conveniently grouped are discussed by WILSON (:**00**), by CALKINS (:**01**), and at some length by CALKINS in a recent article (:**03**); hence we may concern ourselves here mainly with those forms which appear to show nuclear conditions nearest those in Empusa.

SCHAUDINN published in 1894 an account of the division of the nucleolus-like body in the center of the dividing nucleus of Amoeba, and although he recognized that this appeared to play the chief rôle in nuclear division, he reserved, till further comparative studies, his ideas on the mechanical details of the process.

[1]As in this paper I shall have to refer frequently to the figures already published in *plates XIV* and *XV* which accompanied my foregoing paper on *The morphology and development of Empusa* (BOT. GAZETTE **41**: 192–208. March 1906), I have numbered the figures on this plate consecutively with them.

BLOCHMANN ('94) and KEUTEN ('95) first described the division of the centronucleus in Euglena, and the latter author gave an interpretation of the function of the nucleolus, giving to it the name "nucleolo-centrosoma" (p. 219). According to KEUTEN's observations, the nucleolus-like body of Euglena elongates in the prophases of nuclear division, and functions as a kind of spindle, which, however, appears to be solid and homogeneous, and not fibrillar as in the usual type of spindle. Other spindle substance and centrosomes, as well as "pole-bodies," the author could not find. The chromatin forms many chromosome-like bodies, which, after passing through an "equatorial ring" stage, are finally arranged in diverging daughter groups about the elongated axial strand of the nucleolo-centrosome. Just what the relation is between the dumb-bell shaped nucleolo-centrosome and the dividing chromosomes is not made clear in KEUTEN's figures, although he asserts that this axial rod governs the entire process of nuclear division, since it orients the plane of division and since the chromosomes move along it. Whether this intranuclear body functions solely as an active fibrous mechanism for separating the chromosomes, or whether its poles have in addition the properties of centrosomes, are matters which should be more clearly determined before we can make comparisons with the conditions in Empusa.

BOVERI (:**00**, p. 182, note) suggests in this connection that the nucleolo-centrosome of Euglena is probably a concentrated and sharply individualized intranuclear spindle. CALKINS (:**01**, p. 265) further points out what he regards as an analogy existing between such a connecting rod in Euglena and the true fibrous spindle seen in higher forms.

SCHAUDINN (:**00**) has described a type of nuclear division in the sporozoan, *Coccidium shubergi*, parasitic in the intestine of a myriapod, which resembles even more closely that of Empusa. In the growing individual, according to this author, the nuclei divide a number of times, and finally, by a process resembling progressive cleavage, the body is cut up into many uninucleate individuals, which he terms merozoites. The division of the nucleus at this time is by a "primitive mitosis" (p. 230), totally unlike the double division which takes place in later stages, following the fertilization of the egg. The division in the first instance is quite similar to that of Amoeba and

Euglena, and also resembles very closely that in Empusa. The second kind of nuclear division is regarded by SCHAUDINN as still simpler, since centrosomes appear to be wanting entirely. The close resemblance of the latter type to certain division-figures in Empusa suggests, however, that the differences noted by SCHAUDINN may have been more apparent than real, and that poor fixation, due perhaps to the thickness of the membrane about the fertilized egg, may have been the cause for his failure to find the intranuclear centers in these cases also.

According to SCHAUDINN (p. 229), in the first mentioned division the chromatin granules gather in the center of the primary nucleus about a diffuse, slightly refractive substance, which stains less with haematoxylin than the chromatin. There results finally a globular central body, which he calls a karyosome, made up of two substances, plastin and chromatin. Upon the appearance of vacuoles within it, the karyosome grows larger, and it ultimately elongates to form a dumb-bell shaped central core to the dividing nucleus. At this stage the chromatin strands appear to radiate from the poles of the central body, differing in this respect from the corresponding nuclear figure of Euglena. The continued elongation of the central core is accompanied by the further massing of chromatin about the two daughter-halves of the central body, and the nucleus finally assumes a shape comparable to an hour-glass. In the slender connecting strand which unites the diverging nuclear halves there appears a peculiar *Zwischenkörper* which SCHAUDINN regards as probably a thickening of the fibrous strand which connects the halves of the karyosome. After the final constriction into two, the daughter nuclei, without entering upon a period of rest, begin immediately a second division.

While those members of the Entomophthoreae which live parasitically in the bodies of insects have attracted attention for more than a century, only a few investigators have published observations on the coenocytic character of the mycelium of these fungi. MAUPAS ('**79**, p. 252) records having seen many nuclei in the hyphae of *Empusa muscae;* while VUILLEMIN ('**86**) published drawings showing a similar condition in *Entomophthora gloeospora* Vuil. FAIRCHILD ('**97**) also mentions having noted the multinucleate mycelium in certain species of Empusa. BREFELD, who has studied the group

more than any other investigator, has noted also ('84, p. 41) that the hyphae of Conidiobolus, which grows parasitically on Exidia and similar fungi contain many nuclei; but he contributes no comment on the internal structure of Empusa, whose external characters and development he has described in great detail.

CAVARA next published ('99) some cytological observations on *Empusa muscae*, which was shown to have multinucleate conidia, and on *Entomophthora Delpiniana*, with multinucleate conidia; and he showed the importance of this character in delimiting the groups of the Entomophthoreae, a point with which I heartily agree. But CAVARA'S account of the division of the nuclei in these two forms by simple fragmentation is without doubt incorrect, as is plain from the complicated method described in the present paper.

Finally, GALLAUD (:05) has quite recently studied a form, Delacroixia, apparently similar in habit to Conidiobolus, whose mycelium as well as conidia contain numerous small nuclei.

The application of refined technique to the study of the cytology of these organisms has resulted in but one paper—that by FAIRCHILD ('97) on Basidiobolus—which deals exhaustively with the nuclear details. EIDAM ('86), who first discovered Basidiobolus and figured its uninucleate cells, and LOEWENTHAL (:03), both working with unsectioned material, and quite recently WOYCICKI (:04), have also contributed certain cytological observations in their studies on this form.

Basidiobolus shows, as we shall see, little resemblance cytologically to Empusa, and RACIBORSKI ('96) contends that it should not be included in the Entomophthoreae. However, since this form is generally considered in connection with the group, it seems best to review at this time FAIRCHILD'S account of the nuclear division in Basidiobolus. This author has described in great detail the peculiar division by which the two small beak-cells are cut off from the adjoining gametes. The division of the nuclei in these beaks bears little resemblance to that in Empusa, nor, indeed, to the process in any other thallophyte so far described; it rather resembles, according to FAIRCHILD, that in higher plants, in that a cell-wall is laid down through the instrumentality of a cell-plate. During the prophases of division, the nucleole disappears, and the author thinks it is probably used to form spindle

fibers. The nuclear membrane, as in the higher plants, appears to be dissolved and a barrel-shaped or cylindrical multipolar spindle is formed. Strongly staining granules terminate each of the poles of the broad spindle, and in the early phases, the many chromosomes gather in an equatorial plate. In the anaphases, a double row of granules appears in the equator of the spindle, which is regarded by the author as forming a true cell-plate, since the new cell-wall is laid down between them. It should be noted in this connection, however, that such a cell-plate appears to lack the earlier fusion of the fibers, which, in the higher plants, invariably precedes the splitting and the subsequent deposition of cell-wall substance between the two new plasma membranes thus formed. Vegetative nuclear division was also observed by FAIRCHILD, who evidently regards the process as essentially similar to that just described, although in this instance he did not succeed in finding a cell-plate.

WOYCICKI (:**04**), on the other hand, while agreeing with FAIRCHILD in general as to the events of mitotic division in Basidibolus, confirms RACIBORSKI'S assertion (**'99**) that the new cell-wall grows centripetally as a ring-formed growth, like that in Spirogyra, and in this case entirely independently of the spindle.

NUCLEAR DIVISION.

The nuclei in the coenocytic hyphae of Empusa are comparatively large, measuring frequently as much as 7–9 μ in diameter, and are thus especially favorable for a study of the phenomena of nuclear division. In the vegetative hyphae they are usually spherical when in a resting condition; while in conidiophores or in similar elongated cells the nuclei also often become greatly elongated. In the conidiophores of *Empusa* sp. (*figs. 23, 25*[2]), the resting nuclei may even assume irregular and apparently amoeboid shapes.

The resting nuclei of the vegetative hyphae of *Empusa sciarae* (*figs. 19, 27, 57*) have no nucleole-like bodies whatever, whereas in other forms, e. g., *E. muscae* (*figs. 38–40*) and *E. culicis* (*fig. 48*), each nucleus possesses one sharply defined nucleole. In still others, *E. aphidis* (*fig. 44*) and *Empusa* sp. (*figs. 22–26*), the number of nucleoles

[2]Figures numbered 1–48 will be found on *plates XIV* and *XV*. *Figs. 49–67* are on *pl. XVI*, herewith.

varies, since one, two, or sometimes even four such bodies may be normally present in a resting condition. In many cases where these structures do occur, they appear to be surrounded by a clear space, and some show a filamentous connection with the chromatin (*figs. 22, 44*). In other instances (*figs. 47, 48*), no such clear space is seen, but the nucleole appears instead to be closely surrounded by a mass of chromatin.

In optical sections, the nuclei of *E. sciarae* (*fig. 57*) show darker granules which are apparently connected by more lightly staining portions, thus giving an appearance corresponding to the common conception of the chromatin and linin in the resting condition. But careful focusing reveals rather a more or less homogeneous, much convoluted thread, or filamentous material. Since I cannot, in fact, see any appreciable differentiation into chromatic and achromatic portions, I am inclined to regard the chromatin in this instance as resting in the form of a spirem thread.

I am hardly prepared, however, to accept for these nuclei the ideas of VAN WISSELINGH ('99) and of GRÉGOIRE and WYGAERTS ('03), who think that there is no distinction between linin and chromatin. For, though it is true that in the resting nuclei of Empusa the nuclear material appears to be homogenous, during mitosis, on the other hand, some parts retain the stain much more tenaciously than other parts. One may bleach out an iron haematoxylin preparation, for example, until only that portion of the dividing nucleus immediately around the centers remains dark. However, whether this difference brought out by staining is due to mere physical causes, I cannot say.

Resting nuclei take the stain readily and are thus sharply differentiated; whereas those which are in a state of division stain less deeply. Hence in searching for division-stages, one has but to find those nuclei which are lightly stained and from which the color has been more washed out. But this applies apparently only to those nuclei which are somewhat advanced in the process, for such differentiation is not readily noticeable in very young stages. The earliest stages of nuclear division in the two species of Empusa in which I have studied the phenomenon, *E. sciarae* and *E. aphidis*, in fact are not altogether clear. It is to be hoped that other species will prove more favorable for the beginnings of the process. It is not quite clear, for example,

just what events are transpiring in such a nucleus as that figured in *fig. 49*. But from the later condition shown in *fig. 50* to near the close of the telophases, a great abundance of successive stages affords an easy interpretation of most of the events of nuclear division.

It is highly probable that *fig. 49* illustrates the early divergence of the two centers of division present in the middle of the nucleus, although no clew is given in the preparation toward the solution of the puzzling question as to the origin of these centers. In this figure a clearly defined strand connects two darker regions, where, presumably, nuclear material is being aggregated. A clear space, probably a cavity filled with nuclear sap, separates the two centers and encloses the connecting filament. Between *fig. 49* and *fig. 50* is plainly a large gap. In the latter, the two centers are large, conspicuous, intranuclear bodies, from each of which radiate in all directions granular fibers. These fibers appear to connect in some instances midway between the centers with those from the opposite system of fibers; others appear to cross over the equatorial region and to be independent of the other system.

Figs. 51–61 record successively the phenomena attending further divergence of the centers and the massing about them of the material of the divided daughter-halves of the nucleus. It may be noted in these preparations that one of the first evidences of the activity in a nucleus leading to division is the change from a globular to an oval form. During the progress of the internal mitotic changes, the nucleus finally becomes elliptical or oblong and greatly elongated. It may readily be noted also that the long axis of the dividing nucleus corresponds generally with the long axis of the filament. In some instances, however, the nucleus lies obliquely across the hypha, presumably carried about by cyclosis.

An increasing abundance of nuclear sap is shown in *figs. 49–58*. In *fig. 49*, the clear portion is seen to occupy the space between the two diverging centers. From the repeated occurrence of similar nuclei in which the middle appears to be occupied by a clear space, it seems probable that one of the earliest manifestations of mitotic activity in the case of Empusa is the accumulation of karyolymph in the immediate vicinity of the intranuclear centrosomes. In *fig. 50* the nuclear sap apparently lies both between the two centers and in the interstices

between the fibers. In *fig. 51* a clear space is noticed at one side of the dividing nuclear elements, whereas, in the more advanced stages shown in *figs. 52–55*, sap lies mainly between and separating the two active centers of division. In *figs. 54* and *57*, a region almost free from fibrous material separates the daughter halves and gives the appearance of a turgid, intranuclear, vacuolar cavity. It will be noted in these instances as well as in still later stages (*figs. 56, 58, 60*), that this nuclear fluid appears to exert pressure on the chromatic elements, as evidenced by the curved line where the massed chromatic material borders on the vacuolar fluid. In *fig. 56*, which shows the next step in the division of the nucleus following *fig. 57*, the cytoplasm has constricted in two the mother-cavity, and in this figure as well as in the similar stages shown in *figs. 58, 60*, it will be at once noticed that the solid constituents of the young daughter nuclei occupy a pseudosynaptic position, and that the greater part of the cavity of the daughter nucleus is occupied by a clear space. Whether an osmotic pressure of the intranuclear fluid causes this appearance, or whether it is due simply to the massing or contraction within the nuclear cavity of the chromatic elements about the polar centrosomes, can hardly be determined with certainty, but it is probable that both forces are thus operative.

Fig. 58 illustrates an interesting deviation from the more common median constriction of the elongated nucleus shown in *figs. 56, 59, 60*. Here a double cytoplasmic constriction has taken place, resulting in two daughter nuclei and between them a vacuole, which is undoubtedly filled with sap from the mother-nucleus. Probably in this instance the dividing nucleus became so greatly elongated that surface tension operated in such a way that the encroaching cytoplasm constricted it into three parts instead of two, as is usual.

In *fig. 59* is shown an early telophase condition in which the solid constituents of the nucleus are being redistributed throughout the daughter nuclei. Here the movement of chromatic material, as is characteristic for nuclei in this condition, is opposite to that seen in early stages of nuclear division. Whereas in the early phases this material moves toward and masses about the polar center, in the teleophases, it moves in the opposite direction, away from the center. In *fig. 59* the center in each nucleus is still conspicuous, although a

considerable portion of the mass, especially that in the upper daughter nucleus, has moved centrifugally, towards the nuclear membrane. We note also in the upper nucleus of this figure what appears to be a thickening at the outer ends of the radiating filaments, and in the lower nucleus some of the radiations are seen to be double.

I am inclined to interpret *fig. 61* as a very late telophase, and as a near approach to a resting nucleus; between this figure, however, and *fig. 59* there is obviously a wide gap. Such nuclei as that shown in *fig. 61* are comparatively common, however, and without doubt represent a stage in which the center now exists only as a focal region for the attachment of the chromatic fibers to the nuclear membrane. Occasionally one may see at this focal point, especially in preparations stained with the triple stain, a very dimly defined body, apparently a remnant of the old center, lying against the nuclear membrane. But in similar preparations stained with iron haematoxylin, the core of the old center seems to be entirely empty, while immediately around it a dense chromatic mass persists for some time. In *fig. 61*, for example, there remains hardly any visible evidence of the old center of division; a few conspicuous fibers and a darkly stained mass which was accumulated about the center remain, however, to mark its former position, and the fibers now serve apparently to attach the main mass of chromatin to the nuclear membrane.

Apparently such a nucleus is "polarized," at least in so far as there seems to be a special and possibly permanent focal point on the nuclear membrane for the chromatic materials. Whether this is centralized in the same sense as Euglena, or permanently polarized, as in the case of Phyllactinia (HARPER, :05), must be settled by further investigation.

We see that, from very early stages, the centrosomes in these dividing nuclei are conspicuous bodies, which grow larger and more conspicuous as division progresses, and this is due, in my opinion, to the accumulation of nuclear materials about them. Each centrosome is lighter in the middle and has a darker rim (*figs. 50–67*), a phenomenon which I am convinced is partially due to refraction. But careful washing out of the stain sometimes leaves the middle totally bleached out, while immediately around it some parts retain the stain. Each centrosome thus appears possibly to have a core of plastin and a rim of chro-

matin, as is claimed by SCHAUDINN for the similar bodies of Coccidium. *Fig. 61* could therefore be interpreted as showing the rim of chromatin, but the plastin substance of the middle core has entirely disappeared.

The division of the nucleus just described for *Empusa sciarae* takes place in the later vegetative stages, when cross partitions are frequent and when the coenocytic cells are consequently comparatively short. Among the four or five nuclei present in each cell in this condition, we may occasionally find two nuclei in a state of division; generally but one, however, divides at a time. The nuclei in a certain cell do not, therefore, divide simultaneously, but each appears to act in entire independence of neighboring nuclei.

There occurs in earlier stages of the vegetation of the fungus an interesting modification of the process as described above. *Figs. 62–65* illustrate late stages in the division of the nuclei found in long coenocytic cells, in which cross-partitions are few and far apart. It will be remembered that during the earlier vegetative activities of *Empusa sciarae*, nuclear division takes place much more rapidly than cell-division, with the result that septa occur at rare intervals, while, on the other hand, nuclei during this period are abundant. When we come to compare the seemingly different type of nuclear division shown in *figs. 62, 63* with that shown in *figs. 50–55*, we note in each the intranuclear centers and the radiating chromatic filaments mentioned above. But here in the latter type the dividing nuclei assume an hour-glass shape, similar to those of Coccidium as shown in certain of SCHAUDINN'S drawings, instead of the oval or elliptical shape characteristic of the nuclei during the division above described. A careful comparison, however, leads to the conclusion that the only essential difference between the two types of division is in the amount of nuclear sap. In the latter case there is lacking the clear space filled with nuclear sap, between the separating chromatic filaments, so conspicuous in the type above described; or at least the fluid is much diminished in quantity. In *fig. 65* some is still present in the constricted region; but between the separating daughter halves in *figs. 62, 63*, as well as in *fig. 64*, little sap, if any, is evident. *Fig. 65* shows, in fact, a transition between the elongated, hour-glass shaped nuclei of the latter type and the oval ones of the former.

There can hardly be any doubt, especially after we make comparative observations on *Empusa aphidis*, which has a similar type of nuclear division, that such stretched-out nuclei as are shown in these figures get their peculiar form from the currents of protoplasm flowing in these long coenocytic hyphae. Resting nuclei, as is well known, are plastic to a remarkable degree, and thus, in long cells, may frequently become much elongated; so it seems more than probable that these dividing nuclei may likewise become stretched out in the same way.

Figs. 66, 67 represent poorly stained nuclei of *Empusa aphidis* in which division is taking place in a manner evidently similar in every respect to that described above as the second type. Here too we have vegetative hyphae in which septa are few and far apart; hence the general protoplasmic movements must disturb considerably the dividing nuclei. Practically all of the nuclei of this species conform to the type shown in *figs. 66, 67*, for I have but once found a doubtfully elliptical nucleus. The fact that the second type of division alone occurs in the long tubular filaments of *Empusa aphidis* points therefore to the conclusion that the stretching out of the dividing nuclei in these instances is brought about by cyclosis. In this second type of division here described, we can readily imagine that the protoplasmic currents also assist materially in the constriction and final separation also of the halves of the dividing nucleus. We may thus conceive, in the one case, of the protoplasm as undergoing such limited movements on account of its confinement in a short cell, so that the dividing nucleus is but little disturbed, and consequently, by the accumulation of karyolymph, it assumes a short oval or rounded shape; whereas in instances where the cells are long and the protoplasmic currents therefore stronger, the dividing nuclei become drawn out and elongated, and constriction becomes very early evident.

In *figs. 64, 65*, we note an interesting phenomenon. Here occurs an infolding at the poles, giving an appearance as if some stress had indented the nuclear membrane at this point. I have not observed this phenomenon in the oval nuclei of the first type, but it apparently occurs not infrequently in nuclei of the second type. It is possible of course, that the infolding may be an artifact, caused in some manner by the reagents. Such cases furnish indisputable proof, at any

rate, that the intranuclear centers are strongly anchored to that nearest portion of the nuclear membrane situated poleward from them.

We may summarize these results pertaining to the nuclear division of *Empusa sciarae* and *E. aphidis* as follows. During the early stages of division the nuclei become less stainable and slowly change from a rounded to an oval shape. Two diverging centers of division, or centrosomes, become conspicuous near the middle of the nucleus. Fibers may now be seen radiating from the two intranuclear centers, some crossing the median line between the centers, others evidently anastomosing with fibers from the other system. The nucleus elongates still more and the opposed centers, each with its system of radiating fibers, diverge farther and farther apart. The centrosomes appear to increase in size as division proceeds, probably from the aggregation about them of the chromatic material in the radiating fibers.

In cells which are comparatively short, a space filled with sap is early apparent between the diverging daughter masses, as well as in the interstices between the chromatic fibers. This sap increases in amount until in the oval, turgescent nuclei found in such short cells, the middle portion becomes filled with it, and we note a clearer central part, containing at first a few scattered fibers, separating the two polar, darkly-staining regions. On the final withdrawal of the last chromatic filaments to the daughter-poles, the middle of the elongated nucleus becomes perfectly clear and transparent. The cytoplasm now encroaches on the median sap-cavity and, by constriction, cuts the mother-nucleus in two. In some instances, a double cytoplasmic constriction may take place, so that a vacuole filled with nuclear sap is cut off and left between the two daughter-nuclei.

In long cells, on the other hand, or in filaments with few, far-separated septa, the nuclear sap does not accumulate in the manner just described; hence the nucleus, instead of becoming turgid with the liquid secretion, becomes early in the process of division constricted in the middle and greatly elongated, thus assuming the shape of an hour-glass. A few connecting strands in the constricted portions remain for some time, while the active polar regions, with their dense accumulation of chromatic material, become separated farther and farther, with the result that the two daughter-halves are finally pulled apart.

The lack of accumulation of nuclear sap in the latter type of nuclear division constitutes the only difference between this type and the one described above.

The accumulation of sap in the nucleus in the first instance is probably due to the lack of disturbance of the process by the restricted protoplasmic currents in the short cells. The lack of accumulation of sap in the second instance is probably due to the disturbing influences of the stronger protoplasmic movements which undoubtedly take place in the long tubular filaments. In the first type the chromatic substance in the newly formed daughter-nuclei comes to lie in a mass at one side of the nuclear cavity, thus resembling somewhat a synaptic condition. In the other, the nuclear materials of the young daughter-nucleus, massed about the centrosome, are closely enveloped by the surrounding cytoplasm, and not until later in the reconstructive processes which follow, does the nuclear sap appear.

Towards the close of division, the center in each nucleus comes to lie close to, if not actually on, that portion of the nuclear membrane nearest the pole. Its attachment and anchorage to the nuclear membrane is proven by the frequent indentation of the membrane at this point. In the young nucleus the center remains conspicuous for some time, but finally, with the resumption of a resting condition, it becomes entirely lost to view. In the resting nucleus, the nuclear materials appear to be distributed more or less evenly on a much convoluted, seemingly homogeneous, filamentous thread which resembles a spirem.

We have now to emphasize, before entering upon a discussion of the general bearing of these facts, certain peculiarities at once noticeable in this primitive mode of nuclear division. In the type first described, the nuclear membrane plainly persists throughout the whole process of division; also in certain nuclei of the elongated type, it undoubtedly persists (*figs. 65–67*), although it is here not so conspicuous. I am inclined further to regard a membrane as present around the chromatic fibers in *figs. 62–64*, notwithstanding the fact that in the preparations it cannot be seen. The iron haematoxylin stain is probably accountable for the failure to bring out the membrane clearly in this instance. Hence we may record at this point that in the case of Empusa, the nuclear membrane is at least usually persistent through-

out the whole of nuclear division, and that, consequently, the entire process is intranuclear.

Secondly, we note the absence of any definite chromosomes in this peculiar division; and equally noticeable is the failure of the chromatic material to become aggregated into an equatorial plate, as well the want of a definite achromatic spindle. Careful counts, however, of the fibrous strands radiating from the centrosomes indicate the probability of a constant number of these chromatic fibers. I have in many instances counted about sixteen of these radiations from the polar view (*fig. 55*), but it is perhaps impossible to determine exactly the correct number, on account of the great confusion of threads. I believe, nevertheless, that these fibrous strands of chromatic material represent the chromosomes, and further, that the two daughter nuclei each receive an equal number.

There seems little evidence for the existence of a differentiated achromatic spindle, but further study in related species may possibly assist in determining what here may correspond to such a structure. It is true that in *fig. 64* is shown an indefinite, intrafibrillar substance which might be taken for a spindle, but I am convinced that the thickness of the section in this instance is responsible for this misleading appearance. Careful observation reveals chromatic fibers in a lower plane of focus and it is their great number and close proximity in the background that probably causes the indefinite, washed-out appearance between the sharply defined filaments. In *fig. 54* also there is shown a similar substance between the radiating fibers, whereas in *fig. 55* this is not so noticeable. *Fig. 50* as well shows but little nuclear substance other than that in the sharply defined chromatic fibers radiating from the two centrosomes.

Since all the dividing nuclear substance outside the centers is apparently confined to the two systems of filamentous structures radiating from the centers, we must therefore conclude that there is no intrafibrillar spindle-substance. And, since we see also that these radiating strands appear to be chromatic in their staining reactions and not achromatic, the only conclusion which seems possible is that there is no substance in the dividing nuclei of Empusa which can correspond to an achromatic spindle. I am not prepared, however, for such an extreme belief, which would obviously much belittle the

importance of a fibrous mechanism for the accomplishment of mitotic division.

I should prefer to believe that the achromatic spindle substance, probably present only in small amount, is a part of, and inseparable from the deeply staining radiations. Should this be true, then we may conclude that the kinoplasmic spindle-mechanism is bound up closely with the radiating parts corresponding to the chromosomes. Possibly the chromatin is here more nearly a liquid substance than is usual, hence it may diffuse more readily throughout the linin basis, so as to be indistinguishable from the latter. At any rate, I should regard the chromatic filaments radiating from the centrosomes as corresponding in part to the fibers of the more differentiated spindle of higher organisms; and, further, since these mark the paths of the chromatin, they must also correspond to the mantle fibres. In the case of Empusa, so far as studied, there is obviously nothing which can correspond to the central spindle of more complicated nuclei.

CELL-DIVISION.

Cell-division in Empusa, as in many other lower plants, takes place in entire independence of nuclear division, and also apparently remote from nuclear control. There is concerned in the process no such fibrous structure as a cell-plate; since, in fact, no cell-plate is ever formed at the close of the nuclear division described above. Further, cell-division may not take place till long after all division of the nuclei has ceased; hence coenocytic hyphae result.

The branched conidiophores of *E. sciarae* (*figs. 16, 18*), as well as conidia in the process of abstriction (*figs. 28, 30, 31, 36*) furnish especially fine material for the study of cell-division. Examples are also occasionally met with in sections of vegetative hyphae (*figs. 19, 21*). A striking feature of the process as seen in conidiophores and young vegetative hyphae is the fact that in the cleavage of the cell, the new ring-formed partition-wall grows across a wide vacuolar space. In the case of the abstriction of the conidia, on the other hand, and probably as well in older vegetative stages, although I have not as yet seen the phenomenon in the latter instance, the new wall grows through a mass of cytoplasm. *Fig. 18* shows clearly the method of growth progressively inward of the ring-formed septum.

The plasma-membrane which bounds externally the thin primordial utricle has evidently been infolded at this point, thus forming a deep, narrow furrow. The young partition-wall which is being deposited in this groove can not be seen in the figure. We note further in *fig. 18* that the two nuclei which are shown are in a state of rest; in fact, nuclear division does not occur at all during the pre-fructifying period characterized by the formation of conidiophores. And in the same figure we also see that the nuclei are separated by a wide vacuolar space from that part of the cell in which division is proceeding, and that they are joined to the active region only by a narrow cytoplasmic connection. It seems reasonable to suppose that cell-division, in this instance, is a cytoplasmic phenomenon and is merely remotely or indirectly subject to nuclear control. In *fig. 18* it will be noted that the stain is deepest at the inner margin of the cleft, showing that in this innermost region in which the new wall is being laid down, the cytoplasm is densest and most active.

Fig. 19 shows a similar ring-formed septum partly across a young vegetative hypha, at a slightly advanced stage of growth. A bridge of cytoplasm is next thrown across the vacuolar space before the wall is completely formed, as is seen in *fig. 20*. This figure brings out most clearly the region of greatest activity. In the preparation, the stain (iron haematoxylin) was well washed out, so that the cytoplasmic bridge as well as the ring-formed wall are left unstained except at the innermost part of the furrow, where a small black granule is conspicuous. In this dark region the new wall is evidently being deposited. Immediately on the throwing across of the cytoplasmic bridge, the greater turgor of the cell below ordinarily causes the partition to bend outward toward the outer end of the hypha (*fig. 20*). This bending is also quite noticeable after the final completion of the partition wall (*fig. 17*).

A study of these figures might lead to the conclusion that we have here a process exactly similar to that already described for certain other fungi (see HARPER, '99, p. 506), in which the cleavage furrow first cuts across the cell and the wall follows later. One would in fact naturally come to this erroneous conclusion, since every one of the drawings mentioned above, except perhaps *figs. 19, 21*, shows clearly the circular furrow, but no sign as yet of the ring-formed septum.

In these preparations, however, the thin, delicate walls are not at all easy to differentiate. I am convinced that, unlike the cases just referred to, in Empusa a delicate wall grows simultaneously with the cleavage-furrow and not later. The figures which show abstriction of the conidia furnish sufficient evidence for this conclusion. In this case, the process of abstriction takes place essentially like the cell-division described for conidiophores, except that here the cleavage-furrow grows through a mass of cytoplasm instead of through a central vacuolar space. In *fig. 38*, the completed wall separating the conidium from the basal cell of the conidiophore may be plainly seen, since the protoplasm is shrunken away on both sides. But in *figs. 28, 30, 31, 36*, although the cleft itself at the base of the conidium is brought out with diagrammatic clearness, the wall which accompanies it is not so evident. Two reasons may be noted here, however, which are not so apparent in the case of conidiophores, for the conviction that the partition-wall is also present in these instances. The wall which cuts off the conidium, when completed, as was noted above in the case of the newly formed septa in conidiophores, is forced upward by the greater turgor of the basal cell, and here forms a kind of columella within the conidium. While it is possible that the cleavage-furrow itself might be stretched and forced upward in this fashion, yet it is more than likely that the unsupported plasma-membranes bounding the cleft could not withstand the considerable pressure which is developed. A further reason for the belief in the necessity of the cooperation of a ring-formed wall in these instances is seen in the shooting off of the conidia immediately on the completion of their abjunction. In *figs. 29, 37, 43*, are shown conidiospores which have evidently just been shot off and in which the turgescence of the protoplasm has now reversed the position of the cross-wall, making a papilla at the base instead of an indentation. We see clearly in *fig. 37* the delicate wall shrunken away from the spore-plasm. An uncompleted wall at the time of the discharge of the conidium would evidently allow the escape of the protoplasmic contents.

GENERAL DISCUSSION.

It is clear that the division of the nuclei of Empusa which has just been described, although apparently resembling in some respects

amitosis, is certainly much more complicated than a mere mass division such as occurs in the latter process. In the division of the centronucleus of Empusa, we have, as was seen, intranuclear centers of division, or centrosomes, which function as active agents in nuclear division. Centrosomes, when they do occur, are, on the other hand, supposed to take no essential part in amitotic division. In the dividing nuclei of Empusa, we have also, besides active centrosomes, an arrangement of the chromatin in radiating fibers comparable to chromosomes, and, further, a simple spindle-apparaatus. I should therefore separate the process in this form far from amitotic division, although still regarding it as an extremely simple type of mitosis.

In the division of the nucleus in Euglena, the resemblance of the phenomena to amitosis was regarded by KEUTEN as so striking that he called the process in this organism a simple intergradation between direct and indirect division. In the case of Coccidium SCHAUDINN remarks that the division of the nuclei takes place by a "primitive mitosis." Should SCHAUDINN be able to find, further, as is probably possible with improved fixation, the division-centers in his second kind of division, which occurs in the stages following the fertilization of the egg, he should come to the conclusion that he has here also not, as he concludes in his paper, a still simpler type than the first, but a primitive mitosis essentially like the first. For in the event of similar intranuclear division-centers occurring in both cases, he would have two types of division somewhat comparable to the two types mentioned above in Empusa, which, as we have seen, differ from each other only in the amount of nuclear sap present, and in the earlier constriction and elongation of the second type.

In Empusa, Coccidium, and Amoeba, the absence of an arrangement of the chromatin during the prophases of nuclear division in an equatorial plate, attests the extreme simplicity of the mitotic process in these instances. The absence of this equatorial arrangement leaves us, in fact, unfortunately in doubt as to the manner of the equal distribution of the chromatin between the two daughter nuclei. If we accept, however, the commonly accepted doctrine that "the daughter nuclei receive precisely equivalent portions of chromatin from the mother nucleus" (WILSON, :**00**, p. 70), we must conclude

that this equal division of the chromatin occurs somewhere in the obscure prophases; in Empusa, e. g., probably long before the appearance of the conspicuous centers seen in *fig. 50*.

The absence of the arrangement of the chromatin into an equatorial plate prior to the divergence of the two daughter masses possibly results from the poor development of the achromatic spindle, due to the small amount of linin present in the nucleus. To this same cause is probably due also the failure to form definite chromosomes in these simple organisms. In Amoeba, according to SCHAUDINN'S observations ('**94**), there are apparently no radially arranged chromatic filaments; while in Coccidium (SCHAUDINN, :**00**) and Empusa, evidently a still higher type obtains, since in both these instances we have formed, rather late in division, filaments of chromatin, which undoubtedly correspond to the chromosomes, and are radially arranged about the centrosomes.

The formation of an "equatorial ring" in the nuclear division in Euglena, and of a more compact equatorial arrangement of the chromatin in Euglypha (SCHEWIAKOFF, '**88**), Actinosphærium (HERTWIG, '**98**), Paramœcium (HERTWIG '**95**), Aulocantha (BORGERT, :**00**), and other Protozoa, certainly indicates the presence in these forms of a more highly differentiated mechanism for the halving of the chromatin. In all these cases, we note the early formation of chromosomes, which are usually very clearly defined, and generally a well developed spindle, consisting of both central spindle as well as polar mantle-fibers; so that we are justified in the conclusion that in these more highly differentiated figures there is a greater amount of intranuclear achromatic substance present than in the nuclei of Empusa and Coccidium.

We may compare at this point the degree of differentiation of the intranuclear spindle in these organisms. KEUTEN regards the dumbbell shaped nucleolo-centrosome in Euglena as probably serving as a spindle mechanism; and BOVERI (:**00**) and CALKINS (:**01**, :**03**) also think that the strand of connecting substance in this constricted nuclear body corresponds to the central spindle of higher organisms. CALKINS (:**01**, p. 265) points out in this connection that Paramoecium furnishes a clew to the relationship of such connecting strands in Euglena to the fibrillated central spindle, since in Paramoecium the

"central portion of the division-figure is a single strand which widens and becomes fibrillated at the ends." SCHAUDINN (:**00**, p. 229) evidently does not so regard the corresponding portion of the dividing nucleus in Coccidium, since he calls this connecting strand simply "Verbindungsfaden der Tochterkarysome," and says that "von Spindlefasern und Poldifferenzirungen ist keine Spur wahrzunehmen." I am also inclined to believe that no part of the constricted nucleolar body in Euglena and Coccidium is homologous with the central spindle of more complicated nuclei, since in all cases where a structure occurs which can be positively referred to the central spindle, it consists of usually distinct fibers which extend between and connect the diverging chromosomes. In these instances, the connecting portion of the dividing nucleolar body bears no such relation to the chromatic filaments, but instead it lies simply as a slender core in the axis of the mitotic figure. Further, in the centronucleus of Empusa, which is undoubtedly similar in every respect except this one to that of Coccidium, such a connecting body does not occur at all, unless, indeed, it be represented in *fig. 49*. Therefore, the strand connecting the constricted nucleolo-centrosome of Euglena and Coccidium, in my opinion, does not represent, phylogenetically, the central spindle, nor in fact any structure of the higher nuclei, but is a structure which is confined, so far as yet known, to these two Protozoa. It is just what SCHAUDINN calls it, viz., simply a drawn-out filament connecting the daughter centrosomes, which has no apparent function. On the other hand Paramoecium, as shown in HERTWIG'S figures, shows a true central spindle, and the final median constriction of this spindle and the consequent aggregation of the fibers of the middle portion into what appears to be a single strand, does not present a figure which can be in the least compared, as CALKINS claims, with the nucleolo-centrosome described above. If there be any indication at all of central spindle in these simpler centronuclei, then, in my opinion, it must be looked for in the dimly defined, continuous, bluish substance, for example, shown in the drawings of Coccidium (see SCHAUDINN'S *figs. 31, 32*), which lies between the daughter chromatin masses. SCHAUDINN himself, however, evidently believes that these are not spindle fibers. In the case of Euglena, the central spindle is probably represented by the dim

achromatic substance remaining between the separated chromosomes, e. g., in KEUTEN's *fig. 11*. But in Empusa, there is no appreciable achromatic substance in the corresponding equatorial region of *figs. 62, 67*. There is, therefore, according to my interpretation, in the simple cases where no equatorial arrangement of the chromatin takes place, practically no development of a central spindle; but whether these two facts are related somehow as cause and effect must await further investigation. Hence we may regard the intranuclear figure in the case of Empusa and Coccidium, as an extremely simple apparatus, which consists merely of the two opposed centers of division, each with its system of polar radiations. Further, these polar rays must all correspond in function to the mantle-fibers, instead of in part to the extranuclear polar asters of the higher animals, since they all mark the paths of movement of chromatin material. As seen in *figs. 50, 54, 55, 59*, for example, the fact is quite apparent that the intranuculear centrosomes lie some distance from the nuclear membrane, and that there is no appreciable differentiation in the radiations which extend in all directions from them. All appear alike to consist, at least in part, of chromatin material. In later stages, represented in *figs. 64, 65*, the centers appear to have been pulled to the periphery so that they come to lie against the nuclear membrane. I am inclined to think that this peripheral position represents the ultimate fate of all of the centrosomes, since the very last stages (e. g., *fig. 61*) almost invariably show the old centers lying at one side against the nuclear membrane. Such figures lead us to believe that after all there may be a slight differentiation in the astral radiations, since those fibers which attach the centrosome to the nuclear membrane may be mainly concerned in this peripheral movement of the centers, forming in these instances a sort of "antipodal cone" of fibers. At any rate, while there may be, in such a spindle, certain polar structures which appear to have a special function and thus to form an "antipodal cone," there is no such striking differentiation of the aster into a "principal cone" and "polar rays" as was described by VAN BENEDEN.

In those more complicated centronuclei in which the chromatin is gathered during nuclear division into an equatorial plate and in which definite chromosomes are formed, as in Euglypha, Paramoecium, and

other Protozoa, a more or less clearly defined, fibrous, central spindle is found in addition to the mantle fibers. The absence of the central spindle in the simpler type of intranuclear division seen in Amoeba, Coccidium, and Empusa, and its meager development even in more complicated cases, clearly suggest that the central spindle-fibers, when present, play only a minor rôle in nuclear division as maintained by HERMANN ('91), viz., that they are non-contractile supporting elements, which form a basis on which the movements of the chromosomes take place. The chromatic structures in Empusa are undoubtedly moved poleward without the assistance of such connecting fibers, and they seem to be supported entirely by the surrounding nuclear sap.

These facts may be interpreted as thus furnishing a strong argument against the acceptance of the "pushing theory" of DRÜNER ('95), who supposes an active growth or elongation of the central spindle, thus pushing the spindle-poles farther and farther apart; and at least in part against the suggestion of MOTTIER (:03, :04), who thinks that the chromosomes may be conveyed to the poles both by a pushing and a pulling action of the spindle-fibers.

No clear explanation of the mechanism which accomplishes these primitive divisions has yet been marked out. As pointed out above, there are in Empusa no specially differentiated mantle-fibers, since the radiating astral rays of the intranuclear figure themselves mark the paths of the chromatin-movement. Whether the movements which take place in these radiations are similar to those which occur in the aster of the more highly differentiated extranuclear centrosphere, I cannot say, but this seems quite probable. In Empusa the radiations extend in all directions from the centrosome and some are anchored firmly to to the persistent nuclear membrane at its nearest point, while others project into the nuclear cavity, apparently ending free in the karyolymph. Now, a contraction of the radiating fibers would undoubtedly accomplish just the phenomenon which we see takes place. The fibers seem to shorten and to thicken, and an appearance suggesting an accumulation of darker staining material immediately around the centrosome results. The distal indentation of the nuclear membrane which we see occasionally (*figs. 64, 65*) should also be regarded as strong evidence that a pull of some sort or a contraction

of fibers in this region has taken place. But I can see no evidence in this instance of a using up of any of the material which has accumulated about the poles, as has been suggested by STRASBURGER (:00) to explain the shortening of the mantle-fibers in certain cases.

It may be pointed out in this connection that the fact that the fibrillar radiations in Empusa appear to be almost homogenous, and further, chromatic in their staining reactions instead of achromatic, does not seriously detract from the reasonableness of the contractile hypothesis, as applied to this form, since it is only necessary to assume that contractile linin is also present in small amount in the fibers, along with chromatin. WILSON ('95), in fact, maintains that in the case of echinoderm eggs, the fibers are derived not merely from the linin-substance, but also from the chromatin.

As in the telophases of mitotic processes in general, in the later stages in Empusa a centrifugal movement of the chromatin sets in, which may sometimes begin even before the two daughter-nuclei are separated by constriction from each other. *Fig. 59* shows such a late condition, in which the chromatin-movement seems to be of the nature of an active outward growth, since we now note at the distal ends of the fibers accumulations of darker and apparently denser material. Should we assume that the aggregation of chromatin about the centrosome in the first instance is brought about by the contractility of the kinoplasm in the radiations, then we must suppose that later some subtle change occurs in the body of the centrosome itself, or else in the fibrillar rays, to stop contraction and to set up an opposite growth of the fibers. But I should regard it as not an impossible assumption that the centrifugal movement in the latter instance might be brought about simply by a loosening up the chromatin in the increasing nuclear sap by which it is surrounded—a phenomenon which would probably follow as a mere mechanical consequence the final cessation of the forces which caused the centripetal movement.

The suggestion that these alternating centripetal and centrifugal movements of the chromatin are of the nature of flowing movements appears to gain some support in the case of Empusa. MONTGOMERY (:01, p. 352) concludes that this flowing movement of the nuclear materials is automatic; but I fail to see how this author can retain,

even in part as he does, the idea of the contractility of the secondary linin-fibrils, in addition to the above theory, since an automatic movement such as he conceives to take place should be regarded as an amoeboid movement in response to chemotropic stimuli. WILSON (:01, p. 575) also regards the chromatin as "a liquid substance which may be absorbed or given off by an achromatic basis such as plastin or linin, and may thus flow from one part of the nucleus to the other." The latter author appears to adopt to a certain extent the ideas of BÜTSCHLI ('92), in that in his studies on Toxopneustes he has become thoroughly convinced that the astral radiations are in part the result of centripetal currents, or diffusion-currents, of hyaloplasm converging on the centrosphere.

While it is quite possible that the chromatin in Empusa is a liquid substance which may flow or diffuse about through an achromatic linin basis, as WILSON suggests, this, in my opinion, does not preclude the idea of a contractile linin substance serving as the mechanism of mitotic division. I must say, however, that while entirely convincing evidence is lacking that the primitive mitosis in Empusa is accomplished by means of a contraction and a growth of the fibrillar, kinoplasmic radiations, there is, on the other hand, even less evidence in favor of other theories; for example, that the movement of the chromatin is automatic, due to chemotropic forces which are supposed to emanate from the centers; or that this movement is due to diffusion-currents induced by the chemism active at the centers; or that it results from magnetic or electrostatic forces, an idea which has been recently revived by LILLIE (:05).

In the primitive mitotic division characteristic of Empusa, we see but little resemblance to the corresponding process as described for other low plants. In all these cases, even in the Myxomycetes (HARPER, :00), a well-defined spindle and chromosome, and an equatorial arrangement of the chromatin may be observed. It is apparently very common among the thallophytes that the nuclear cavity and membrane persist during a large part of the mitotic processes; see, for example, figures of Erysiphe (HARPER, '97), of Albugo (STEVENS, :01), of Dictyota (MOTTIER, :00). But in all of those thallophytes in which centrosomes occur, the latter are *extra*- and not *intra*-nuclear bodies. Empusa is therefore in this respect unique

among the thallophytes and may be regarded as a primitive form; and further, the fact that it possesses intranuclear centers of division may perhaps be regarded as adding another point in favor of HERTWIG's ('98) view as to the intranuclear origin (by the extrusion of centrosome) forming substances from the nucleus of the extranuclear centrosomes of the more highly differentiated organisms.

It has been already pointed out above that Basidiobolus, which has been generally regarded as a member of the Entomophthoreae, shows in its mitotic features (its broad, multipolar spindles, and its formation, according to FAIRCHILD ('97), of a cell-plate), as well as in other morphological aspects, wide differences from Empusa.

Cell-division by means of the growth inward of a ring-formed wall is apparently a common type of division among the filamentous thallophytes. Such a constriction of the cell has so far been shown for Beggiatoa (HINZE, :01), the blue-green algae, Ulothrix (DIPPEL '65), Spirogyra (STRASBURGER,'80), Cladophora (DAVIS,'04), the red algae, and a few other forms. WOYCICKI (:04), contrary to FAIRCHILD'S ('97) assertions, contends that the cell-wall in Basidiobolus also is a centripetal growth. The gametes of Sporodinia and the conidia of Erysiphe are cut off in a similar manner, except that, according to HARPER ('99), the ingrowth here is simply a deep narrow furrow and not the growth inward of a ring of fungus cellulose. The wall in this case is deposited later between the two plasma-membranes.

As has been shown in this study of Empusa, the ring-formed cleavage-furrow starts at a definite region of the plasma-membrane, sometimes remote from the nuclei; and further, the nuclei at no time appear to be concerned, directly, in the process. TOWNSEND'S ('97) observations on nucleated and enucleated fragments of protoplasm leave no doubt, however, as to the ultimate necessity of the presence of a nucleus, in order to initiate the cytoplasmic activities in Empusa which lead to cell-division. Whether the localized stimulus in this case results first in a deposit of a ring of cellulose-substance on the inner surface of the wall of the mother-cell, which might then by its growth progressively inward be regarded as the agent of cleavage; or whether there first occurs in this region an infolding of the plasma-membrane, thus resulting in a circular furrow, to be soon followed by a deposit of wall-substance in the cleft, I am not able to state. In either

case, at any rate, the importance of the plasma-membrane as a factor in such a cell-division should be emphasized. The process in Empusa, in fact, would seem to furnish an argument in favor of NOLL'S (:03) view that in Bryopsis the controlling factor of embryonic growth is located in the *Hautschicht*. Apparently in Empusa a definite region of the plasma-membrane is stimulated to action, a ring-formed infolding of the membrane occurs, and at once in the cleft thus produced, the new wall begins to be deposited. A darker accumulation, presumably of kinoplasm, may now be seen at the inner margin of the cleft (*figs. 18, 20*), where the activities leading to the cleavage of the in-pushing plasma-membrane and to the ingrowth of the partition-wall are evidently greatest. But the plasma-membrane does not alone seem to be the active agent for these phenomena, for *fig. 18* shows a darker portion, having appreciable thickness, which apparently marks a more or less broad region of concentration of kinoplasm. This fact, therefore, may be regarded as an argument against the plasma-membrane itself being the sole controlling factor in this case. Further, MOTTIER'S ('99) experiments on Spirogyra and Cladophora, in which, by reason of the disturbance due to centrifugal force, the cellulose-ring, when once begun, was never brought to completion, notwithstanding the fact that the plasma-membrane was still intact, furnishes very convincing evidence against the acceptance of the theory that the *Hautschicht* alone is the controlling factor of wall-formation in these instances.

The fact that the cleft and ring-formed wall are finally carried across a wide vacuolar space (*figs. 17–21*), will not permit of the application to this case of SWINGLE'S (:03) explanation for the mechanism of the cleavage in Rhizopus, Phycomyces, and other forms. For it seems impossible to conceive how local contractions of the cytoplasm could cause the constriction of the cell in the case of Empusa. We could perhaps think of such a contraction as initiating the process, but that these forces could obtain after the narrow diaphragm of cytoplasm had begun to be pushed across the central vacuole, seems to me inconceivable.

In certain instances in Empusa, as, for example, when a germ-tube is formed (*figs. 8, 9*), the end cell of a filament keeps cutting itself off from behind, thus enabling the body of the protoplasm to

travel forward, so to speak, and to seek a favorable environment in which to grow. BREFELD ('83) seems to think, in the case of the similar phenomenon in Ustilago, that the cells which are thus cut off behind are empty, and that in this way no protoplasm at all is wasted in the process. If this were true, a cell-wall would be formed from a single plasma-membrane, thus differing from the division described above, in which the membrane is split so that the wall is deposited between the two. But the apparently empty cell retains its turgescence for a time before collapsing, thus proving that there is at least a film of protoplasm present. Further, sections of similar conditions in which conidia are cut off from a basal shooting-cell (*figs. 28, 36, 38*), show clearly the thin primordial sheath of enucleate protoplasm in the lower cell. The fact that the protoplasm of these lower cells seems to undergo speedy degeneration contributes another point in favor of the idea of the vital importance of the nucleus in nutrition.

I am at a loss to understand why the conidium should be regarded by THAXTER ('88, p. 143) as a one-spored sporangium, since in all the sections of conidia which I have examined there is no sign of a second inner wall. It may be that the plasma-membrane of the plasmolyzed contents of a conidium may have been mistaken for a wall; or, again, it is possible that this author's *figs. 320, 321* represent conidia still surrounded by the slimy protoplasm which is sometimes discharged from the ruptured basal cell.

I wish in conclusion to express my hearty thanks to Professor R. A. HARPER for the privileges afforded in his laboratories; to Professor W. S. MARSHALL, for assistance in the determination of insects; and to the Carnegie Institution of Washington, for a research assistancy under which this work has been done.

SUMMARY.

1. *Life history.*—The life history of *Empusa sciarae* may be summarized as follows: The disease attacks both larvae and adults of the host, Sciara, causing ultimately their death. The young, uninucleate germ-tubes, after they have entered the body-cavity of the insect, grow there at the expense of the nutrient fluids. After the protoplasm has increased in amount, a branching, coenocytic myce-

lium is produced, which in early stages is few septate; later, however, at the culmination of vegetative activity, septa are abundant and branching becomes more frequent.

Finally, the body-cavity becomes almost completely filled with the mycelial filaments, vegetative activity ceases and the death of the insect ensues with the beginning of the fructifying condition.

Radial branches, which mark the beginnings of the conidiophores, are put forth from the short, 3–5-nucleate cells which make up the mature mycelium; in this species, but one branch grows from each cell. These radial hyphae bore their way out through the body-wall of the insect; some form the rhizoids which attach the host to the substratum, while others grow into branched conidiophores. Each conidiophore is cut up by cell-division into uninuclueate segments, each of which pushes out beyond the surface of the host and cuts off from its tip a single uninucleate conidium. The basal cell below the conidium comes to possess but a thin, enucleate primordial utricle, and it finally becomes greatly swollen from the absorption of water. Ultimately this swollen basal vesicle bursts in a ring at the top where it joins the conidial wall, or the columella-like wall may be split in some instances, and the conidium is thus shot violently away, the slimy protoplasmic contents of the lower cell being frequently carried along with the conidium and serving to stick the latter to the substratum. The partition which cuts off the conidium is at first curved upward by the greater turgescence of the vesicle; but when the spore is shot off, this reverses its former position, and in the conidium it appears as a prominent papilla.

2. *Nuclear division.*—The nuclei of Empusa are "centronuclei," since the centrosomes which are active during division are permanently intranuclear.

The division of the nuclei which takes place during the vegetative stages appears to be of the nature of a primitive mitosis, similar in many respects to that described for certain of the simpler Protozoa. The nuclear membrane generally persists during the whole process. A simple intranuclear figure is formed, which in later stages consists of the two opposed centers of division, to each of which converges from all sides a system of fibrous radiations. The many radiations which converge at the two poles correspond to the chromosomes; and,

although they appear to be chromatic in their staining reactions, they probably are made up principally of chromatin, and a small amount of linin. The chromatin at first concentrates about the centrosomes, which thus appear to have a darker rim about a lighter center. The centripetal movement, as well as the later centrifugal movement characteristic of the telophases, may be regarded as of the nature of the flowing or diffusion of a liquid chromatin through a contractile linin basis.

In the nuclear division of *Empusa sciarae*, the chromatin does not appear to pass through an equatorial plate stage.

We may distinguish two shapes of the dividing nuclei in Empusa: one found in short cells of the vegetative hyphae, in which the nuclei in the later stages of division assume an oval or ellipsoidal shape; and another found in long cells, in which the nuclei become themselves greatly elongated and early assume a constricted, hour-glass shape. In the oval nuclei, the nuclear sap accumulates so that the cavity becomes turgescent; while in the elongated nuclei, the liquid does not accumulate, at least not to such an extent as in the first instance, so that the consequent encroachment of the cytoplasm between the two daughter-halves results in an early constriction. In the long cells, cyclosis is doubtless stronger than in the short cells, thus bringing about in such instances a greater disturbance of the mitotic processes.

3. *Cell-division.*—Cell-division in *Empusa sciarae* is accomplished by means of the growth inward, from the wall of the mother-cell, of a ring-formed partition. In a majority of cases, the new cell-wall is carried across a wide, central, vacuolar space; when the older cells become filled with cytoplasm, however, and later when the conidium is abstricted, the wall cuts through the protoplasm which fills the cell. A ring-formed cleavage-furrow starts at a definite region of the plasma-membrane, and a wall is at once deposited in the cleft. A region of some thickness at the inner margin of the cleft, where the processes are most active which lead to the cleavage of the in-pushing plasma-membrane and to the deposition of the partition wall, stains darker than the surrounding cytoplasm. This fact is made the basis for the conclusion that the split plasma-membrane is not the sole active agent of cell-division, although it may be

a controlling factor in the process. Cell division, in this instance, is regarded as a cytoplasmic phenomenon, since the nuclei may be remote from the place of constriction; and, further, they appear to have nothing directly to do with the process. The ultimate necessity of the presence of a nucleus, probably as a controlling factor of nutrition, is proven, however, by the early death of the enucleate basal cells cut off from the conidia and from the end-cells of germ-tubes.

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EXPLANATION OF PLATE XVI.

The drawings were made with the aid of an Abbe camera lucida, together with the Zeiss 2 mm apochromatic obj. N. A. 1:30, combined with compensating ocular 12; except *fig. 16*, which was drawn with compensating ocular 18.

FIGS. 49–65, *Empusa sciarae*. All ×1500, except fig. 64, which is ×2250.

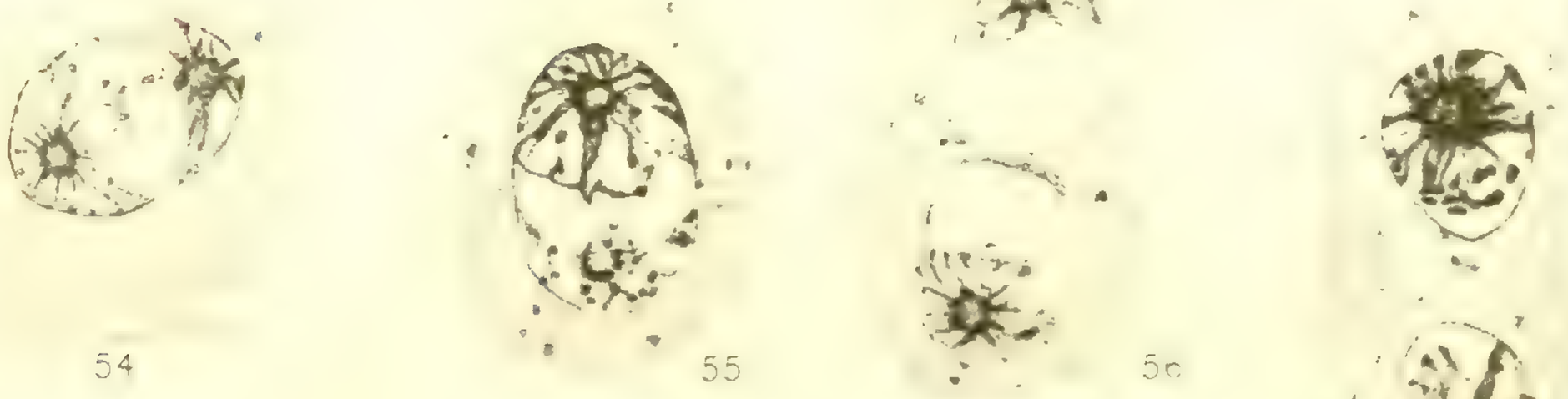
FIG. 49. Probably an early prophase of division.

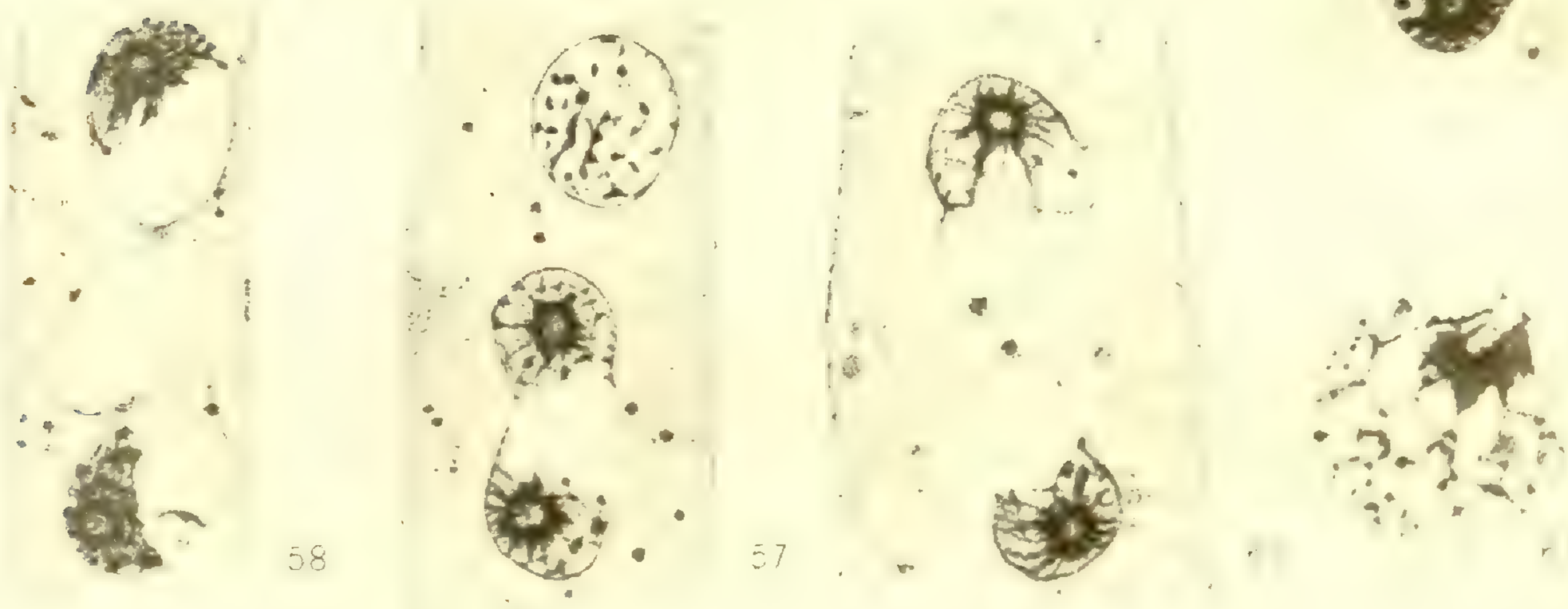
FIG. 50. A considerably advanced stage of division.

FIG. 51. A poorly differentiated preparation in which the karyolymph has accumulated at one side of the dividing nucleus.

FIG. 52. A thin section of an anaphase.

FIG. 53. A similar preparation.





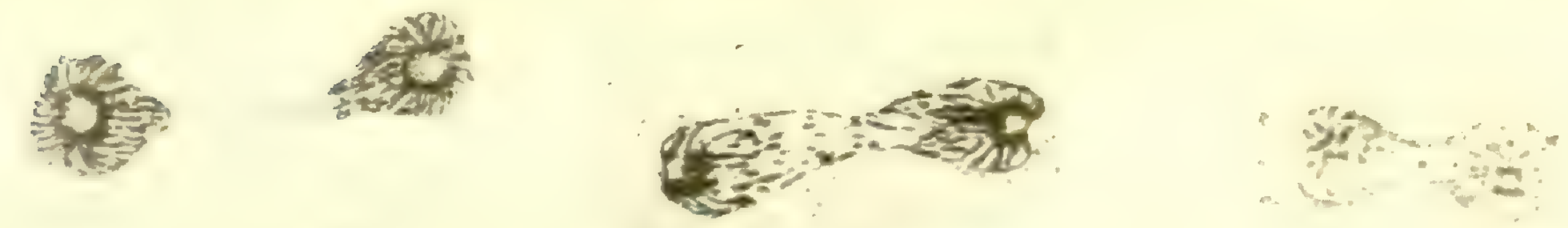


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FIG. 54. A stage in which the daughter-halves appear to be pressed upon by the turgescent, vacuolar middle portion.

FIG. 55. A partly polar view of an obliquely placed nucleus, showing about 16 fibrillar radiations extending from the centrosomes.

FIG. 56. A late condition in which the daughter-nuclei have just been separated from each other by a cytoplasmic constriction. The nuclear contents occupy a pseudo-synaptic position.

FIG. 57. Showing in the upper portion of the hypha a resting nucleus and in the lower, a late stage of nuclear division.

FIG. 58. A double cytoplasmic constriction has taken place so that a vacuole filled with nuclear sap is left between the two daughter-nuclei.

FIG. 59. A telophase condition.

FIG. 60. Another telophase condition.

FIG. 61. Probably a very late telophase, in which the dark rim of the center has survived, whereas the achromatic centrosome-portion has disappeared.

FIG. 62. A late condition of nuclear division which is characteristic of elongated cells.

FIG. 63. A somewhat later stage, also from an elongated cell.

FIG. 64. A younger stage, in which the nuclear membrane has been indented at the ends of the nucleus distally from the poles.

FIG. 65. An elongated nucleus in division, in which one pole shows an indentation.

FIGS. 66, 67, *Empusa aphidis*. ×1500.

FIG. 66. A poorly stained preparation, showing the division characteristic of the elongated cells of this species.

FIG. 67. An earlier stage of division.

BIOLOGICAL RELATIONS OF DESERT SHRUBS.

II. ABSORPTION OF WATER BY LEAVES.

V. M. SPALDING.

DURING a study of certain shrubs growing in the vicinity of the Desert Botanical Laboratory near Tucson, Arizona, it has been found that the leaves of some of them absorb water, while those of others do not. Although leaf absorption is treated by leading physiologists as a matter of indifference, or at any rate of secondary importance, it has seemed worth while to inquire whether differences of habit in this particular, on the part of these desert plants, may not be correlated with other characteristic peculiarities; if so, even if the fact should turn out to be of small importance physiologically, it may be significant from a biological point of view.

Our knowledge of leaf absorption as yet is fragmentary and uncertain. For the general subject it is quite unnecessary to cite the voluminous and contradictory literature. DANDENO[1] has given a useful historical résumé, reference to which and to paragraphs in BURGERSTEIN'S more recent work[2] is sufficient for the present purpose. In regard to various highly modified plants, however, the case is quite different. SCHIMPER has made such detailed observations of certain epiphytes as to leave no doubt that they normally absorb large quantities of water through their aerial parts, and that this is a distinct physiological advantage, or even necessity. In view also of investigations cited by BURGENSTEIN it becomes necessary to accept the fact of leaf absorption in the case of various other plants.

As for the plants of arid regions, the evidence has been less conclusive than could be wished. VOLKENS, in his classical work, describes various special structures by means of which, presumably, many of the plants of the Egyptian-Arabian desert take up dew

[1] DANDENO, J. B. An investigation into the effects of water and aqueous solutions of some of the common inorganic substances on foliage leaves. Trans. Can. Inst. 7:238. 1901.

[2] BURGERSTEIN, A., Die Transpiration der Pflanzen. 1904.

through their leaves, but the woody species growing in the arid territory of the southwestern United States are so different in their habits and in their environmental relations as to preclude the settlement of the question for them, even within the bounds of probability, in any other way than by direct observation and experiment; in fact they are found, as regards leaf absorption, to differ widely among themselves. The object of the present paper, therefore, is the presentation of such facts as have been determined for a limited number of species indigenous to southern Arizona.

In this region existing physical conditions give to the question special interest. As is well known, precipitation is meager, except at relatively high altitudes, and is distributed throughout the year, with no distinctively rainy season. The rainfall, moreover, is extremely uncertain, and for months at a time is often so slight that it does not wet the soil for more than a few centimeters, an amount of precipitation likely to be of very little positive advantage as far as root absorption is concerned. Under such circumstances, in which delicate adjustment is the condition of survival, it would seem that plants capable of leaf absorption might have a distinct advantage in times of prolonged drouth, during which occasional showers occur which are too light to penetrate the soil. As will be seen, however, only a limited number of species appear to enjoy this advantage to an appreciable extent.

Nearly all of the species selected for investigation grow in the immediate vicinity of the Desert Laboratory. A single one, *Holacantha Emoryi*, which seems not to be indigenous here, was obtained from the grounds of the University of Arizona. The following classification of the plants employed into biological groups is provisional, but will serve to direct attention to the very diverse ecological history of the species now growing together in this region.

BIOLOGICAL CLASSIFICATION OF PLANTS STUDIED.

I. Shrubs, with relatively slight modification of form and structure, their habits plainly indicating mesophytic origin. Celtis, Covillea, Lycium.

II. Shrubs or small trees, more conspicuously modified, but retaining manifest traces of mesophytic habits. Parkinsonia, Prosopis, Acacia.

III. Woody or partly herbaceous plants, exhibiting peculiar modifications of distinctly xerophytic types. Fouquieria, Holacantha, Koerberlinia, Zizyphus, Atriplex.

IV. Plants of the most pronounced xerophytic character. Opuntia, Cereus, and other cacti.

V. Plants adapted by habit, rather than structure, to desert conditions. Sphaeralcea and many other half-shrubby or more or less herbaceous forms.

Of the species employed in the experiments, *Celtis pallida* is a shrub, growing commonly to the height of one to one and one-half meters on the laboratory hill, where it is rather abundant. It holds its foliage so well that it might be ranked as an evergreen, though it suffers to some extent from the effects of frost. Its leaves are rough-hairy, thin but firm in texture, and conforming in general to the generic type. *Covillea tridentata*, the well-known creosote bush, is the most abundant woody species of this region. Its small coriaceous leaves, presented more or less edgewise to the sun and covered with waxy varnish, are well protected against excessive transpiration. *Lycium Berlandieri* is a small shrub, more than a meter in height, of frequent occurrence on rocky exposures. These species, of the three genera named, while well adapted to their habitat, exhibit characters far less conspicuously xerophytic than those of many of the plants with which they are associated.

Coming to the second group, *Parkinsonia Torreyana* attains the dimensions of a small tree, and is conspicuous by reason of its green bark, from which it has the common name of *palo verde*. Though a denizen of the desert, it is not a dry ground form, but frequents low places, where more water is available than on the mesa or even on the adobe soil of the hills, where *Parkinsonia microphylla*, a related species, does well. *Prosopis velutina*, the mesquite, grows chiefly in low ground, within reach of abundant water, but it also occurs, though scattering and undersized, on the adobe soil of rocky hills. Like the *palo verde* and many other leguminous plants, the leaves of the mesquite exhibit in their structure and position excellent adaptations for the prevention of excessive transpiration. *Acacia constricta*, of similar distribution, occurs on the mesa and also on rocky upland. It is a vigorous shrub,

one or two meters in height. These several species of Parkinsonia, Prosopis, and Acacia thrive well under the rather severe conditions to which they have become accustomed; they all retain, however, manifest traces of mesophytic habits, particularly in their choice of habitat.

The species assigned to the third group, among which are *Fouquieria splendens*, *Holacantha Emoryi*, *Koerberlinia spinosa*, and *Zizyphus lycioides*, present more striking modifications of form and structure than do any of the members of the preceding groups, and, though differing greatly among themselves, agree in possessing such conspicuous adaptations to xerophytic conditions as easily to rank next to members of the following biological group.

The cacti are commonly taken to represent the extreme type of xerophytes, but notwithstanding various striking features common to members of this order, there are essential differences of habit and adaptation, even between closely related species, rendering it quite impossible to generalize from the study of "typical forms" in the investigation of biological problems presented by them.

The half-shrubby and herbaceous plants are much like those of other regions, exhibiting as a rule no structures that would be thought of as distinctively xerophytic, but accommodating themselves to desert conditions by their habits, especially such as enable them to take advantage of periods favorable for rapid development and production of seeds.

By way of first ascertaining whether any of the plants of these several groups absorb enough water through their leaves or internodes to be readily detected by weighing, the following method was employed: A small branch with leaves functionally active, though often showing plainly the effects of long drouth, was severed and the cut end immediately covered with vaseline. In a few instances, which are specified, branches without leaves were used. The branch was then weighed and directly afterwards immersed in water, except at the cut end, for a definite time, usually about three hours. At the end of this period, after exposure to the open air long enough to be certain that the surface was fully dry, the branch was again weighed, and the increase of weight, if any, was

taken to represent closely the amount of water absorbed, though, owing to loss during the operation of drying the surface, the amount absorbed must often have been rather greater than the increase of weight indicated. In the first preliminary set of observations, a pair of large balances, weighing satisfactorily to ten milligrams, was employed; but in subsequent experiments quantitative balances were used, the weighing being made to a milligram in each case. Changes during the process of weighing rendered it as useless as it was unnecessary to attempt a higher degree of accuracy.

Inspection of Table I shows that leafy shoots of Celtis, Covillea, and Lycium, by immersion in water for three hours, gained 1.9 to 5 per cent. of their original weight; Atriplex in a little longer

TABLE I.

PRELIMINARY TEST OF CAPACITY FOR ABSORPTION. *November 1904.*

Species	Date	Time	Weight in grams	Gain or loss per cent.
Covillea tridentata (1).....	Nov. 1	10:12 A. M.	18.540	
		1:12 P. M.	19.250	3.8 gain
Covillea tridentata (2)	" 9	10:55 A. M.	8.235	
		1:56 P. M.	8.650	5.0 "
Celtis pallida.....	" 1	10:57 A. M.	24.920	
		1:57 P. M.	26.000	4.3 "
Lycium Berlandieri.....	" 1	10:45 A. M.	7.105	
		1:45 P. M.	7.240	1.9 "
Acacia constricta.....	" 1	11:14 A. M.	6.260	
		1:14 P. M.	6.352	1.5 "
Prosopis velutina.....	" 1	10:30 A. M.	4.200	
		1:30 P. M.	4.250	1.2 "
Parkinsonia microphylla.....	" 1	10:22 A. M.	10.410	
		1:22 P. M.	10.490	0.8 "
Parkinsonia Torreyana.....	" 9	11:03 A. M.	4.070	
		2:02 P. M.	4.085	0.4 "
Atriplex canescens.....	" 17	10:21 A. M.	2.140	
		1:44 P. M.	2.250	5.1 "
Zizyphus lycioides.....	" 9	11:39 A. M.	19.270	
		3:25 P. M.	19.320	0.3 "
Fouquieria splendens.....	" 9	11:21 A. M.	21.770	
		2:24 P. M.	21.920	0.7 "
		3:32 P. M.	21.770	
Koerberlinia spinosa.....	" 10	10:41 A. M.	8.650	
		1:40 P. M.	8.670	0.2 "
Encelia farinosa.....	" 9	12:00 M.	5.010	
		3:00 P. M.	5.320	6.2 "
		3:18 P. M.	5.000	
Sphaeralcea pedata	" 9	12:07 P. M.	1.120	
		3:12 P. M.	1.180	5.4 "
		3.21 P. M.	1.110	

period gained 5.1 per cent.; Parkinsonia, Prosopis, and Acacia gained 0.4 to 1.5 per cent.; and Zizyphus, Fouquieria, and Koeberlinia, all without leaves, showed almost no appreciable gain. Species of Sphaeralcea and Encelia gained in weight 5.4 and 6.2 per cent. respectively, but promptly lost all they had gained by a few minutes drying.

It will be noticed that of the plants employed in this preliminary work those without leaves absorbed no water to speak of, while those in leaf fell into two categories, those absorbing and those not absorbing water in quantity. The experiment, therefore, pointed to leaves rather than internodes as agents of absorption, and indicated, apart from Sphaeralcea, Encelia, and the peculiar Atriplex, only the woody species belonging to the first group as likely to prove capable of absorbing much water.

Starting with the suggestions derived from these facts, a more careful and detailed study was undertaken. Cut shoots were still employed for a time, though it was understood that confirmation of results would necessitate the use of entire plants, and these were, as a matter of fact, employed to a large extent in the later work. Care was exercised in the selection of material, and in each case its source and any conditions liable to affect results were noted.

GROUP I.

Celtis pallida.

Four specimens of this species were selected, all in good condition, though apparently not as active physiologically as they would have been earlier in the year. Numbers 1 and 2 were fresh shoots, while numbers 3 and 4 were small branches taken from older bushes. Those numbered 1 and 3 were cut so as to include a large leaf surface as compared with the other two. In every case the cut ends were covered at once with vaseline, and the first weighing was made as soon as practicable after bringing them to the laboratory. They were then wet at frequent intervals for a little more than three hours, and, after drying the surface, were weighed again, after which they were left in the laboratory to dry until the next day, when the same steps were repeated. Finally they were immersed in water over night and again weighed.

TABLE II.

CELTIS PALLIDA. *December 1904.*

No.	Date	Time	Weight in grams	Loss or gain per cent.	Period of treatment
1	Dec. 19	10:44 A. M.	1.834		
		1:55 P. M.	1.853	1 gain	After wetting nearly 3 hrs. 11 min.
	20	9:48 A. M.	1.516	18.2 loss	" drying 19 hrs. 53 min.
		2:10 P. M.	1.592	5 gain	" wetting nearly 4 hrs. 22 min.
	21	10:35 A. M.	1.895	19 "	" " " 20 " 25 "
2	19	10:54 A. M.	1.392		
		2:02 P. M.	1.410	1.3 gain	After wetting nearly 3 hrs. 8 min.
	20	9:57 A. M.	1.192	15.5 loss	" drying 19 hrs. 55 min.
		2:25 P. M.	1.238	3.9 gain	" wetting nearly 4 hrs. 28 min.
	21	10:52 A. M.	1.440	16.3 gain	" " " 20 " 27 "
3	19	11:13 A. M.	2.137		
		2:20 P. M.	2.185	2.2 gain	After wetting nearly 3 hrs. 7 min.
	20	10:07 A. M.	1.734	20.6 loss	" drying 19 hrs. 47 min.
		2:38 P. M.	1.870	7.8 gain	" wetting nearly 4 hrs. 31 min.
	21	11:05 A. M.	2.182	16.7 "	" " " 20 " 7 "
4	19	11:25 A. M.	1.505		
		2:31 P. M.	1.521	1.1 gain	After wetting nearly 3 hrs. 6 min.
	20	10:16 A. M.	1.351	11.2 loss	" drying 19 hrs. 45 min.
		2:46 P. M.	1.387	2.7 gain	" wetting nearly 4 hrs. 30 min.
	21	11:15 A. M.	1.535	10.7 "	" " " 20 " 29 "

Inspection of Table II shows:

1. That all four specimens absorbed water very slowly just after they were freshly cut, and that the rate of absorption was greatly increased after they had lost weight by remaining over night in the dry air of the laboratory.

2. The rate of absorption showed a correlation with extent of leaf surface, being considerably greater in the two specimens with large extent of leaf surface than in the other two.

3. The weight lost by drying for a given period was nearly or quite regained when the leaves were given a full supply of water for a corresponding length of time. The capacity of this species for leaf absorption, under the conditions described, is thus fully demonstrated. Its deportment in the seedling stage, which offered for experiment perfectly fresh and unmutilated material, will next be considered.

Seedlings of *Celtis pallida* were grown from seeds sown November 14, 1904. When used for experiment in January and February,

1905, they were all in healthy condition, and when taken up were found to have fine vigorous roots. In addition to the cotyledons, which were still capable of photosynthesis, each seedling had two or three perfectly healthy green leaves that had attained the length of about one centimeter. The seedlings were transplanted into earth contained in glass vials of convenient size for accurate weighing, sheet rubber being used to prevent evaporation from the soil.

In the case of seedling number 1 the earth was very moist when the rubber was adjusted, and it was found that this plant, which was transpiring vigorously, showed almost no capacity for absorption. The case was different with seedling number 2, which was left some five days after transplanting with the soil open to the air, so that it became relatively dry before the rubber was adjusted. The cotyledons of number 1 were removed, their place of attachment being carefully covered with vaseline; number 2 had one large cotyledon which remained in place during the experiment. These details are necessary to an understanding of the different behavior of the two seedlings as shown by Tables III and IV, which cover the period from January 21 to February 1, at which latter date the experiment was concluded.

It is seen that both seedlings transpired regularly and largely, but that number 1, in spite of the fact that its transpiring surface had been lessened by the loss of its cotyledons, exhibited a decidedly higher rate of transpiration than number 2, which was in drier

TABLE III.

CELTIS PALLIDA. SEEDLING NO. 1. *January 1905.*

Date	Time	Weight in grams	Loss or gain	Conditions
Jan. 21	1:19 P. M.	26.256		
	4:05	26.229	0.027 loss	After standing in dry air
23	10:15 A. M.	26.094	0.135 "	" " " " "
	2:50 P. M.	26.061	0.033 "	" " " " "
25	10:50 A. M.	25.936	0.125 "	" " " " "
26	9.50	25.870	0.066 "	" " " " "
27	9:28	25.806	0.064 "	" " " " "
	2:53 P. M.	25.810	0.004 gain	After wetting
	3:41	25.803	0.007 loss	" standing in dry air
28	11:02 A. M.	25.762	0.041 "	" " " " "
	1:33 P. M.	25.764	0.002 gain	" wetting
	2:58	25.759	0.005 loss	" standing in dry air

TABLE IV.

CELTIS PALLIDA. SEEDLING NO. 2. *January and February 1905.*

Date	Time	Weight in grams	Loss or gain	Conditions
Jan. 30	10:09 A. M.	22.638		
	1:10 P. M.	22.630	0.008 loss	After standing in dry air
	3:40	22.626	0.004 "	" " " " "
31	9:45 A. M.	22.653	0.027 gain	" wetting
	12:45 P. M.	22.628	0.025 loss	" standing in dry air
	3:13	22.634	0.006 gain	" wetting
Feb. 1	9:48 A. M.	22.592	0.042 loss	" standing in dry air
	9:50	0.027		Weight of plant above ground
	2:08 P. M.	0.045	0.028 gain	After wetting

soil, an interesting result in harmony with earlier experiments, showing the direct relation between available soil water and rate of transpiration.[3]

On the other hand, while the quantity of water absorbed by number 1 was so meager as to be negligible, that absorbed by number 2 was much more, in one case almost exactly 100 per cent. of its own weight, i. e., of the part above ground when it was afterwards severed from the root. Number 2, although apparently perfectly healthy while the work was in progress, seems nevertheless to have reached a condition in which the diminished supply of water from the soil was followed by a marked acceleration of leaf absorption, while in the case of number 1, growing as it was in moist soil, no such compensation was made or required.

Of interest as bearing on the validity of determinations of absorption by the use of detached shoots is the fact that while seedling number 2, after it had finally been cut off at the surface of the ground, absorbed in a few hours its own weight of water, it had done precisely the same thing before mutilation, only in longer time. It may well be that a detached shoot, cut off from its normal source of water supply, will absorb more rapidly through its leaves than the same shoot, which, while attached, is supplied, even inadequately, from the soil; but this difference plainly does not justify the degree of discredit that has been thrown upon evidence derived from experiments with separated parts of plants.

Three other seedlings of *Celtis pallida* were treated like the preceding ones, except that the observations were not begun until

[3] SPALDING, V. M., Soil water in relation to transpiration. Torreya **5**:25. 1905.

the plants had been some weeks in the vials to which they were transplanted, and weighings were made during a longer period and with more complete records as to soil conditions, health of seedling, etc. Their records as to weight are given in Tables V, VI, and VII. All of these seedlings were in a healthy condition and apparently capable of entirely normal development. The small extent of surface through which absorption and transpiration took place renders the consistency of the results all the more striking. In every case water was absorbed when it was presented to the leaves and internodes, and transpiration was resumed as soon as their surfaces were dried.

TABLE V.

CELTIS PALLIDA. SEEDLING NO. 3. *February 1905.*

Date	Time	Weight in grams	Loss or gain	Conditions
Feb. 14	11:20 A. M.	23.084		Weight of seedling and outfit
	2:25 P. M.	23.091	0.007 gain	After immersion in rain water
15	9:40 A. M.	23.044	0.047 loss	" standing in dry air
	11:55	23.053	0.009 gain	" immersion in rain water
16	11:42 A. M.	23.007	0.046 loss	" standing in dry air
17	2:11 P. M.	23.046	0.039 gain	" immersion in rain water
20	10:23 A. M.	22.895	0.151 loss	" standing in dry air
	2:51 P. M.	22.916	0.021 gain	" immersion in rain water
21	10:28 A. M.	22.932	0.016 "	" " " " "
	12:00 M.	22.907	0.025 loss	" standing in dry air
	3:02 P. M.	22.887	0.020 "	" " " " "
22	12:08	22.835	0.052 "	" " " " "
	12:11	22.828		Weight after removing cotyledon
	2:14	22.835	0.007 gain	After immersing in rain water
	2:34	0.035		Weight when cut at surface of earth
	2:38	22.799		" of vial, rubber, and earth
25	1:58	0.015		" of plant above ground, air-dried in laboratory

When the first weighing was made, February 14, seedling no. 3 had two cotyledons, still attached, and three foliage leaves. The cotyledons showed some indications of drying. The earth in the glass vial in which the seedling was growing was becoming rather dry, but still contained sufficient water to maintain a transpiration current for a week and probably longer. On February 16, the note was made "one of the cotyledons drying, curled, and getting stiff; the other paler than the foliage leaves, but still flexible, otherwise the seedling is in good condition."

On February 22 the cotyledons were removed and the subse-

quent deportment of the seedling indicates that their failing condition previous to removal may be disregarded as not materially affecting the results. As the table shows, the weight of the whole plant above ground, including cotyledons, was less than the weight of water transpired in 19 hours (Feb. 14–15) and also less than the gain of weight by absorption in 26 hours (Feb. 16–17), a conclusive proof of the relatively large quantities of water absorbed and transpired by this seedling during the period of experimentation. The facts regarding seedlings 4 and 5 are so fully set forth in Tables VI and VII as to render further explanation unnecessary.

TABLE VI.

CELTIS PALLIDA. SEEDLING NO. 4. *February and March 1905.*

Date	Time	Weight in grams	Loss or gain	Conditions
Feb. 14	3:30 P. M.	20.130		Weight of plant and outfit
15	10:02 A. M.	20.090	0.040 loss	After standing in dry air
	12:47 P. M.	20.093	0.003 gain	" immersion in rain water
16	12:00 M.	20.031	0.062 loss	" standing in dry air
17	3:11 P. M.	20.051	0.020 gain	" immersion in rain water
20	10:46 A. M.	19.821	0.230 loss	" standing in dry air
	3:19 P. M.	19.825	0.004 gain	" immersion in rain water
21	10:54 A. M.	19.830	0.005 "	" " " " "
	12:11 P. M.	19.813	0.017 loss	" standing in dry air
22	12:29	19.696	0.117 "	" " " " "
	3:08	19.707	0.011 gain	" immersion in rain water
March 2	10:04 A.M	19.080	0.627 loss	" standing in dry air
	2:04 P. M.	19.085	0.005 gain	" immersion in rain water
	2:25	19.080	0.005 loss	" standing in dry air
	2:30	0.070		Weight when cut off at surface of earth

TABLE VII.

CELTIS PALLIDA. SEEDLING NO. 5. *February 1905.*

Date	Time	Weight in grams	Loss or gain	Conditions
Feb. 20	11:16 A. M.	20.207		Weight of plant and outfit
	3:28 P. M.	20.204	0.003 loss	After wetting with rain water (not immersing)
21	11:09 A. M.	20.219	0.015 gain	After immersion in rain water
	12:15 P. M.	20.214	0.005 loss	" standing in dry air
22	12:34	20.172	0.042 "	" " " " "
	3:18	20.178	0.006 gain	" immersion in rain water
	3:33	0.025		Weight when cut off at surface of earth
25	2:11	0.012		Weight after air drying

Covillea tridentata.

The specimens of creosote bush selected for experiment were taken from four different sources, for the sake of securing material as different as practicable in regard to the amount of water in the tissues. Number 1 was from a bush growing near an irrigating ditch, where it had been abundantly supplied with water. Its leaves were large, dark green, and fresh, and numerous flower buds had been formed. Number 2 was from a plant growing on the mesa a few rods distant. Its leaves were smaller and lighter green, and in comparison with number 1 it was plainly a dry ground form, though it did not have the appearance of having suffered to any great extent from lack of water. Numbers 3 and 4 were from plants growing on the mesa, near the foot of the laboratory hill, where in a dry time the Covillea, the only shrub that keeps alive there, shows the effects of drouth very badly. Their leaves were still smaller and paler in color, those of number 4 especially, indicating by their minute size and other peculiarities a plant that had long lacked a sufficient supply of water. The contrast between this and the first member of the series was very striking. It should be stated, however, that none of the specimens were in quite so dried-up a condition as those employed early in November before the December rains, which though meager—0.82 inch (21^{mm}) thus far—had freshened vegetation to some extent. The dried-up leaves that were dying in November had been shed, and the leaves remaining on the bushes when the experiment was conducted, late in December, were apparently in a vitally active condition.

It will be noticed by reference to Table VIII that, precisely as in the case of Celtis, all the specimens of Covillea gained very little in weight as the result of wetting soon after they were cut. Number 1, from the irrigated bush, gained least, and number 4, from the dry ground plant, gained most. After prolonged drying and again wetting, the gain was much greater than before, the greater gain in each case being made by number 4, which, as already stated, was from the most distinctively dry ground form.

The deportment of number 1, from the robust, well-watered bush, is instructive, especially as it may throw light on the question as to whether leaf absorption is a normal process that takes place

TABLE VIII.

COVILLEA TRIDENTATA. *December 1904.*

No.	Date	Time	Weight in grams	Loss or gain per cent.	Period of treatment
1	Dec. 26	11:05 A. M.	2.529		
		2:17 P. M.	2.538	0.4 gain	After wetting nearly 3 hrs. 12 min.
	28	10:32 A. M.	1.957	22.9 loss	" drying 44 hrs. 15 min.
		2:18 P. M.	1.996	2.0 gain	" wetting nearly 3 hrs. 46 min.
	29	10:50 A. M.	2.188	9.6 "	" " " 20 " 32 "
2	26	11:12 A. M.	2.434		
		2:35 P. M.	2.453	0.8 gain	After wetting nearly 3 hrs. 23 min.
	28	10:40 A. M.	2.234	8.9 loss	" drying 44 hrs. 05 min.
		2:33 P. M.	2.258	1.1 gain	" wetting nearly 3 hrs. 53 min.
	29	11:03 A. M.	2.416	7.0 "	" " " 20 " 30 "
3	26	11:21 A. M.	2.261		
		2:58 P. M.	2.281	0.8 gain	After wetting nearly 3 hrs. 37 min.
	28	10:48 A. M.	1.980	13.2 loss	" drying 43 hrs. 50 min.
		2:45 P. M.	2.020	2.0 gain	" wetting nearly 3 hrs. 57 min.
	29	11:14 A. M.	2.225	10.1 "	" " " 20 " 29 "
4	26	11:28 A. M.	2.646		
		3:03 P. M.	2.688	1.6 gain	After wetting nearly 3 hrs. 35 min.
	28	10:55 A. M.	2.357	12.3 loss	" drying 43 hrs. 52 min.
		2:56 P. M.	2.415	2.5 gain	" wetting nearly 4 hrs. 1 min.
	29	11:23 A. M.	2.766	14.5 "	" " " 20 " 27 "

under natural conditions. This shoot, with its large, fresh, turgid leaves, lost water by drying approximately twice as rapidly as did those from dry ground, with their much smaller leaves and firmer tissues, and on subsequent wetting absorbed far less than the latter in proportion to previous loss. Unlike these, moreover, the leaves of the first specimen, in the course of alternate drying and wetting, lost their fresh look and became discolored. The impression was received that this specimen, taken from a perfectly fresh plant and requiring no additional supply of water, suffered pathological changes in the course of the treatment to which it was subjected, while the others, coming from dry ground plants in need of water, absorbed it as by a perfectly normal process. Even these, however, were not in a condition for rapid leaf absorption when first cut, their gain per cent. being decidedly less for a given period than that exhibited by individuals of the same species during observations made before the December rains. In brief, the experiments of December 26–29, in connection with those of November 1–9, indicate on the part of the creosote bush marked capacity for

subaerial absorption after protracted drouth, but more limited capacity for such absorption, even if artificial drying is resorted to, when it is receiving a better supply of water.

Lycium Berlandieri.

At the time when the observations on Lycium were made, late in December, most of the summer leaves had fallen and fresh ones, following recent light rains, were only beginning to appear. Consequently it was difficult to secure entirely satisfactory material, but a few specimens were finally obtained for experiment which were in a normal and active condition. The leaves of this species are small, only about one centimeter in length, but otherwise the plant gives the impression, as already stated, of having retained up to the present time distinctively mesophytic tendencies. The rapidity with which the leaves were transpiring was at once obvious when weighing was undertaken, and, as in cases previously cited, absorption was found to take place extremely slowly while the leaves were still fresh. Even after drying, water was absorbed in no case as rapidly as it had been lost. Thus number 2 lost 3 per cent. of its weight by drying three and one-half hours, and gained afterwards by wetting nearly four hours 1.9 per cent. Of the actual capacity of this species for subaerial absorption the experiments leave no room for doubt; but the specimens employed deported themselves much like the well-watered Covillea, except that no suggestion of pathological change in the course of the treatment to which they were subjected was noted. When gathered they were simply in the condition of fresh, actively transpiring plants,

TABLE IX.

LYCIUM BERLANDIERI. *December 1904.*

No.	Date	Time	Weight in grams	Loss or gain per cent.	Period of treatment
1	Dec. 28	10:15 A. M.	3.065		
		1:47 P. M.	3.076	0.4 gain	After wetting nearly 3 hrs. 32 min.
	29	10:15 A. M.	3.235	5.2 "	" " nearly 20 hrs. 28 min.
		1:42 P. M.	2.956	8.7 loss	" drying 3 hrs. 27 min.
		3:25	3.002	1.6 gain	" wetting nearly 1 hr. 43 min.
2	28	10:22 A. M.	3.122		
		1:56 P. M.	3.028	3.0 loss	After drying 3 hrs. 34 min.
	29	10:00 A. M.	2.802	7.5 "	" " 20 " 04 "
		1:53 P. M.	2.855	1.9 gain	" wetting nearly 3 hrs. 53 min.

which apparently could derive no advantage from an additional supply of water presented to their leaves.

The record of these three species of Celtis, Covillea, and Lycium has been given at length, on account of the importance of establishing beyond doubt the fact that in these plants, which have been taken to represent desert species that retain in structure and habits obvious indications of mesophytic origin, leaf absorption certainly takes place, and apparently as an entirely normal process. We have next to deal with a group of species genetically related, which deport themselves quite differently from members of the first biological group in regard to leaf absorption. As representatives of this second group, species of Parkinsonia, Prosopis, and Acacia were selected, all belonging to the Leguminosae. The record of experiments and their results is such as to admit of statement in few words.

GROUP II.

Parkinsonia Torreyana.

The specimens of *palo verde* employed in this work were seedlings some two months old. One was cut about thirteen hours, the other (number 2) an hour and a half before weighing. After weighing an attempt was made to wet the leaves by repeatedly immersing the seedlings in water. The experiment might fairly have been dropped at this point, since, as it was found impossible to wet them, leaf absorption could hardly be thought of; but as there remained a possibility of some slight absorption where drops of water collected on the surface of the youngest parts, the attempt was continued with number 1, which was repeatedly immersed during a period of something over three hours.

As seen from Table X this seedling, so far from gaining by absorption of water presented to it, actually lost 1.8 per cent. of its weight in three hours and thirteen minutes, its surface having remained almost entirely unwetted, so that loss of water was possible during the whole, or nearly the whole, of this period. Seedling number 2 was allowed to dry, after an unsuccessful series of attempts to wet its surface. Its loss of weight, as might be expected, was greater than that of number 1.

If these results are compared with those of November 1 and 9, derived from similar experiments with shoots of *Parkinsonia micro-*

TABLE X.

PARKINSONIA TORREYANA. SEEDLINGS. *December 1904.*

No.	Date	Time	Weight in grams	Loss or gain per cent.	Conditions
1	Dec. 31	9:32 A. M.	0.961		
		12:45 P. M.	0.944	1.8 loss	After repeatedly immersing in water
2		9.37 A. M.	0.886		After drying, following repeated attempts to wet the surface
		12:52 P. M.	0.855	3.5 loss	

phylla and *P. Torreyana*, the conclusion must be drawn that the species of Parkinsonia represented here either absorb no water, or at most an exceedingly small quantity, through their leaves. Before making the experimental test it was thought that the fresh, rapidly transpiring leaves of seedlings might exhibit a capacity for absorption not shown by those of older plants, but this has not proven to be the case.

Prosopis velutina.

Work on the mesquite was carried on at intervals for a number of weeks in January, February, and March, the material first employed being obtained from mature specimens, while in the later experiments seedlings were used. Of specimens taken from mature plants only the leaves were immersed in water. In some cases the upper surface resisted wetting, while in others both surfaces were easily wetted. This was followed, as indicated by some increase of weight, by absorption of water in limited quantities. The seedlings which were employed in subsequent experiments remained unwetted in all cases when they were immersed in water, and in spite of the fact that two of the specimens had been left to dry as much as forty-two hours and showed the effects of this treatment before immersion, there is no evidence that they absorbed any water whatever.

It is apparent, then, that as long as the leaves of the mesquite are perfect and resist wetting they absorb no water, even after drying for some time, but that they may absorb more or less after they have become old and can be wetted. It is very questionable, to say the least, whether in the latter case this process has any physiological significance. It would seem that in the mesquite, as in the *palo verde*, adaptations to xerophytic conditions have been carried so far in the direction of preventing excessive transpiration that leaf absorption, as a normal process, does not take place.

Acacia constricta.

A series of experiments with this species was carried out, but it was found unfavorable for exact results, owing in part to the fact that its leaflets become tightly closed after wetting, thus rendering it difficult to secure perfect drying of the surface without overexposure and consequent uncertainty as to the true weight. Accordingly, the conviction that the data obtained were unreliable led to their rejection. For this second group, therefore, we are restricted to the positive results obtained from Parkinsonia and Prosopis, which exhibit either no capacity or very slight capacity for leaf absorption, so long as the leaves are in perfect condition and normally active.

GROUP III.

This third group includes representatives of a number of genera much modified in form and structure, and differing among themselves in their methods of meeting desert conditions. Several of these are more commonly seen without than with leaves, photosynthesis then taking place in their green shoots; while others, more dependent on leaf activity, are commonly in a leafless condition during a large part of the year, pushing out new leaves promptly when conditions are favorable, and dropping them again when they become adverse, as is seen particularly in the case of Fouquieria.

Holacantha Emoryi.

Of this peculiar shrub a small branch with leaves was cut and left several hours to dry. At the end of this time it was still fresh, with no indication of wilting. After weighing it was wet for two hours and thirty-nine minutes, after which it was weighed again, the weight remaining unchanged. Leaving the shoot now to dry until the next day, and then wetting it for four hours and twelve minutes, there was a gain in weight of only 0.5 per cent. Part of the same shoot, destitute of leaves, was treated in the same way, and, after wetting four hours and eleven minutes, also showed a gain of barely 0.5 per cent. of its former weight. These results indicate on the part of this species capacity for leaf absorption so inconsiderable that it may be neglected.

Koerberlinia spinosa, a closely related species, agrees with Holacantha as far as observations have yet been made. Only leafless

branches have been obtainable, but these, as in the preceding species, are green, and for a large part of the year the plant has no other organ of photosynthesis. So far, then, as present evidence goes, absorption through leaves or internodes is not to be predicated of either of these plants.

Zizyphus lycioides.

After the preliminary experiments already recorded, very little satisfactory material for the study of this species was obtainable, as the plant cast its leaves and remained bare until after the period of study was concluded. From observations made early in the year, however, it appears that leafless shoots of Zizyphus do not absorb water in appreciable quantity, but that leafy shoots have considerable absorptive capacity, indicating that it is the leaves and not the internodes through which absorption takes place.

Fouquieria splendens.

Leafy shoots of the *ocotillo*, as shown by Table XI, absorb considerable water when wet for some time after drying. As in various other cases, the loss of weight on drying the shoots after wetting is considerably more rapid than the preceding gain by absorption.

TABLE XI.

FOUQUIERIA SPLENDENS. *January 1905.*

No.	Date	Time	Weight in grams	Loss or gain per cent.	Conditions
1	Jan. 26	12:00 M.	1.614		In each case loss followed drying and gain followed wetting the sp-eci mens during the periods indicated in the time column.
	27	10:00 A. M.	1.582	1.9 loss	
	28	11:40	1.664	5.2 gain	
		3:20 P. M.	1.598	4.0 loss	
	30	1:02	1.518	5.0 "	
		3:28	1.530	0.8 gain	
		4:09	1.523	0.5 loss	
	31	4:15	1.490		
	Feb. 1	1:32	1.585		
2	Jan. 26	2:31 P. M.	4.214		
	27	10:15 A. M.	4.112	2.4 loss	
	28	11:45	4.059	1.3 loss	
	30	2:04 P. M.	4.363	7.5 gain	
		3:04	4.255	2.5 loss	
		4:02	4.197	1.4 "	
	31	11:42 A. M.	3.761	10.0 "	
		2:45 P. M.	3.840	2.1 gain	
		3:45	3.798	1.1 loss	

It appears, then, that for this biological group, which includes a number of plants for the most part unrelated systematically, no general rule can be formulated regarding leaf absorption. The experiments go to show that Holacantha and Koerberlinia hardly absorb at all, while Fouquieria is certainly capable of absorbing considerable quantities of water.

GROUP IV.

The only representatives of the cacti that have been studied thus far are two species of Opuntia, *O. Engelmanni* and *O. versicolor*. A number of specimens of each species were selected after a prolonged drouth, the results of which were plainly seen in their much shrunken condition, very favorable, it would seem, for the demonstration of absorption if this ever takes place. As the material was rather bulky the large balances were employed, a terminal joint in each case being severed and weighed; but there is no reason to doubt the substantial accuracy of the results.

As shown by Tables XII and XIII, *Opuntia Engelmanni* did not in any case gain more than 0.6 per cent. of its original weight, even when immersed in water upwards of 45 hours, and it is safe to conclude from this result, drawn from experiments with a number of specimens, that the species in question does not normally absorb any considerable quantity of water in this way. *Opuntia versicolor*, on the other hand, treated in the same manner, showed

TABLE XII.

OPUNTIA ENGELMANNI. *December 1904.*

No.	Date	Time	Weight in grams	Loss or gain per cent.	Conditions
1	Dec. 3	10:15 A. M.	82.360		In each case gain followed wetting and loss followed drying during the periods indicated in the time column.
		1:30 P. M.	82.850	0.6 gain	
	5	11:00 A. M.	83.360	0.6 "	
2	6	10:12 A. M.	72.085		
		1:10 P. M.	72.470	0.5 gain	
		3:05	72.080	0.5 loss	
3		10:39 A. M.	131.750		
		1:33 P. M.	132.290	0.4 gain	
		3:20	131.760	0.4 loss	

TABLE XIII.

OPUNTIA VERSICOLOR. *December 1904.*

No.	Date	Time	Weight in grams	Loss or gain per cent.	Conditions
1	Dec. 3	10:30 A. M.	16.390		
		1:55 P. M.	16.770	2.3 gain	See Table XII
	5	11:11 A. M.	16.780	0.1 "	
2	6	9:55 A. M.	10.640		
		12:14 P. M.	10.850	2.0 gain	
		3:02	10:630	2.0 loss	
3		10:30 A. M.	8.115		
		12:35 P. M.	8.360	3.0 gain	
		3:10	8.115	2.9 loss	

a gain of 2 to 3 per cent. The specimens most shrunken with drouth were found to absorb water most rapidly.

The rapid loss of water and the curiously close correspondence in each case between the percentages of gain and loss, suggest that in this species it is merely the tubercles that act as organs of absorption, and notwithstanding the fact that the water absorbed is so promptly given off in a dry atmosphere, it appears probable that in a period of frequent light rains the continued absorption of water by the tubercles is precisely the necessary preparation for the development of the young shoot which presently follows. Meantime the different deportment of these two species of Opuntia as regards amounts of water absorbed, corresponding as it does with a marked difference in size of their tubercles, suggests the desirability of a more extended comparison of these structures in different cacti with reference to their capacity for absorption and the physiological value of the process.

GROUP V.

A discussion of the annuals and partly herbaceous perennials that have been referred to a fifth biological group, many of which, structurally at least, are not to be thought of as characteristic desert plants, does not fall within the limits of the present study. As already stated, as many of them as have been studied agree in promptly absorbing water when it is presented to their leaves and internodes, which, however, is given off so rapidly in dry air that it hardly seems possible that its absorption is of any utility. *Cf.* Table I, Encelia and Sphaeralcea.

SUMMARY.

From the preceding observations and experiments, in which woody plants were chiefly employed, it has been seen that certain species of desert plants of southern Arizona absorb water presented to their leaves and internodes, while others do not. The species represented in the vicinity of the Desert Botanical Laboratory may be divided into several biological groups, based primarily on the water relation, of which leaf absorption is a phase. Thus, in the first group, including shrubs, which retain well marked mesophytic tendencies, leaf absorption is characteristic. Members of the second group, more distinctively xerophytic in various structural particulars, are incapable of leaf absorption during their period of normal activity. The third group, decidedly xerophytic, but including species of widely different structure and habits, exhibits corresponding differences in regard to subaerial absorption, which takes place in some of its representatives and not in others. The fourth group, including cacti which are assumed to represent the extreme type of xerophytes, also exhibits interesting differences in size and structure of the tubercles by means of which water is absorbed. Finally, members of a provisional fifth group, which in habit and structure are nearer than any other others to the mesophytes of moist temperate regions, absorb water largely, but very quickly give it up again.

It may be doubted, perhaps, whether this classification, based on biological relations, has in itself any permanent value, but meantime it serves to express and emphasize what is apparently no mere theoretical conception, but a simple historical fact, namely, that differences of habit on the part of these desert plants, as well as the structural adaptations with which they are correlated, have become established step by step together, during the long period of geographical changes through which the land they now occupy has been passing. A discussion of the physiological significance of the facts which have been brought out does not fall within the province of this paper.

DESERT BOTANICAL LABORATORY,
Tucson, Arizona.

NEW SPECIES OF CALIFORNIAN PLANTS.

ALICE EASTWOOD.

(WITH TWO FIGURES)

Zygadenus exaltatus, n. sp.—Bulb large, pear-shaped, covered with brownish, membranous coats, about 10^{cm} long, and half as wide: radical leaves forming a conspicuous bunch sheathing the base of stem, 6^{dm} or more long, 2^{cm} wide, veins prominent, midrib conspicuous on lower part of leaf, less so above, glabrous except for the short, rather thick cilia on the margin: stem tall and stout, hollow, $7–8^{dm}$ high, 1^{cm} in diameter at base, leafy to the inflorescence: upper leaves merging into the bracts, lower cauline with broad clasping base, $3–4^{dm}$ long and as broad as the radical leaves: inflorescence paniculate, $2–3^{dm}$ long, the upwardly spreading branches varying in length at different stages of development, the lower branches generally bearing only staminate flowers, the perfect flowers principally borne on the main stem above the branches; staminate racemes with peduncles shorter than the bracts; bracts attenuate; bractlets white, membranous, longer or shorter than the slender pedicels, ovate-attenuate: perianth 17^{mm} in diameter, outer divisions sessile, elliptical, obtuse, the gland 2^{mm} from the base with parallel veins below forming a margined claw, veins on the upper part proceeding from the teeth of the gland, branching; inner divisions of the perianth with claw 2^{mm} long, 1^{mm} wide, the gland oblong, obtuse, veins as in the outer division; filaments broadening towards the base, 5^{mm} long, attached to base of perianth, anthers oblong, becoming explanate in age: fruit becoming 2^{cm} long, including the rostrate styles, tapering a little more at base than at summit.

Type collected by *F. E. Blaisdell* at Prindle's ranch, above Mokelumne Hill, Calaveras County, California, at an altitude of 425^{m}, in April 1900. The other specimens in the Herbarium of the California Academy of Sciences are Amador, California, May 1886, *M. K. Curran* (specimen with root); Soda Creek, Tulare County, *C. A. Purpus*, June 1896, no. 1549; Hindeman's Trail over Coyote Pass, Tulare County, California, July 19, 1903, collected by myself. I also saw it on the trail on the way to Little Kern. In habit of growth and

size of bulb it is related to *Z. paniculatus*, but it has flowers much larger and all the floral organs differently shaped. It is probably the largest species of Zygadenus known. It is probable that there is no *Z. paniculatus* on the western slope of the Sierra Nevada.

Silene deflexa, n. sp.—Stems several from a creeping rootstock, about 2^{dm} high, erect, glandular-puberulent especially above: radical leaves spatulate, $1–2^{cm}$ long including the margined petioles, rather thick in texture, obtuse; cauline leaves 3–4 pairs, the uppermost very small, not more than 3^{mm}, the lowest oblanceolate to oblong, obtuse, 2^{cm} long, connate-clasping and nodose at base: flowers solitary in the lower leaf-axils, pedicels erect and close to stem, flowers curved-deflexed in anthesis, erect in fruit; terminal flowers few, cymose, pedicels capillary, $7–12^{mm}$ long, generally shorter than the flowers; calyx narrowly cylindrical in flower, enlarging and breaking apart with the expanding capsule, 10-ribbed, 9^{mm} long, divisions rounded at apex, oblong, sometimes uneven, membranously margined, 1.5^{mm} long; petals with the claws united tc the stamens and the stipe of the ovary at base, woolly, gradually enlarging to the blade, not auricled, blade 4-cleft into linear lobes, the two middle 2^{mm} long, 0.5^{mm} wide, the two lateral narrower and shorter, appendages oblong, reaching the base of divisions, retuse with one side pointed; stamens and styles apparently not surpassing the petals, the latter three and the upper thick part of the ovary splitting into three valves, lower part of ovary thin cylindrical, all together 4^{mm} long; stipe 1^{mm} long and almost as thick: seeds suborbicular, brown, strophiolate.

Type collected in the Hudsonian Zone above the lakes, Canyon Creek, Trinity County, California, by *Vernon Baily*, August 25, 1899.

It is related to *S. Lemmoni*, but differs in the foliage, the shorter filaments and styles, the close inflorescence, and the differently shaped floral organs. It really resembles that species only in having the flowers pendent and the blades of the corolla with four divisions.

Silene lacustris, n. sp.—Cespitose from creeping rootstocks, slender, erect, $1–1.5^{dm}$ high, glandular-puberulent throughout especially the inflorescence, flowering from the lowest axils: radical leaves narrowly oblanceolate, acute or obtuse, tapering to a long margined petiole, all together 2^{cm} long; cauline leaves 2–3 pairs, linear, obtuse, connate-clasping at base, $1–2^{cm}$ long, $1–2^{mm}$ wide:

calyx broadly cylindrical, 1^{cm} long, thin, with ten purple nerves, the divisions rounded, 2.5^{mm} long, 2^{mm} wide; petals with claws woolly and cohering round the thick stipe, gradually broadening to the membranous laciniate auricles, 4^{mm} at top, blades purple, 2-cleft with rounded divisions, the lateral teeth short or none, the appendages bifid and laciniate, 1^{mm} long; stamens and styles exserted; ovary oblong, 4^{mm} long: fruit unknown.

Type collected by the author at Monarch Lake near Mineral King, Tulare County, California, July 21, 1903.

This species belongs to the group of high mountain Silenes, including *S. Grayi*, *S. Watsoni*, and *S. Suksdorfii*. In appearance and shape of leaves it most closely resembles the first, but differs from this and the others in the broader auricles, and the bifid, laciniate appendages of the corolla.

Silene pacifica, n. sp.—Perennial, with thick woody rootstocks; stems generally several, erect, 4.5^{dm} high, viscid throughout, especially the inflorescence, nodes prominent: leaves rather thin, radical and lower cauline oblanceolate to obovate or elliptical, tapering at base and decurrent on the long petiole; blade $5–6^{cm}$ long, $2–3^{cm}$ wide, slightly ciliate, sparingly pubescent, obtuse or acute; petioles margined, dilated and sheathing at base; cauline leaves connate-clasping at base, $4–5^{cm}$ long, the uppermost leaves lanceolate, sessile: flowers verticillate in the axils or cymose on short peduncles, pedicels $0.5–2^{cm}$ long, the longest equaling the longest floral leaves: calyx truncate at base, tubular, becoming somewhat turbinate with the enlarging capsule, very viscid, prominently green or purplish veined, the divisions deltoid, obtuse or acute, 4^{mm} long, green with white or purplish membranous margins, entire calyx 1.5^{cm} long; corolla claret color, the claws of the petals white or tinged with claret, 1.5^{cm} long, attenuate at base and broadening at truncate summit to 4^{mm}, exserted 5^{mm}, blade deeply cleft, each part entire, laciniate, or bilobed, the prominent divaricate teeth on each side almost as long as the divisions, more than 1^{mm}, appendages laciniate-dentate, 1.5^{mm} long; stamens monadelphous at base, encircling the stipe and pubescent, glabrous above, varying in length: pod 11^{mm} long, the tips of the three valves stellately reflexed and often splitting into five or six over the calyx when the seeds are ripe; stipe stout: seeds light brown, slightly pitted, reniform, 2^{mm} wide.

Type collected by the author along the south side of Rodeo Lagoon, not far from the ocean, Marin County, California, July 4, 1905. The species seems to be isolated, as the number of plants is small and it is not elsewhere to be found in the region. It is also in danger of extermination on account of the improvements that are now going on in the vicinity of the military post.

This is most closely related to *S. grandis* Eastwood from Bodega Head, likewise a maritime species. It differs in having claret-colored flowers, a differently shaped calyx, the simpler inflorescence, thinner and different leaves, and entire lack of the velvety pubescence so noticeable on *S. grandis*. A smaller and more slender plant.

Horkelia mollis, n. sp.—Stems several, ascending from the sheathed caudex, red-purple, villous with fine silky spreading hairs, about 2^{dm} in height: radical leaves $6–9^{cm}$ long, less than 1^{cm} wide, the petiole less than half the entire length, often with a few scattered simple leaflets near the base; leaflets crowded towards the top, pinnately divided but apparently pedate on account of the lower divisions surpassing the upper, the divisions linear-spatulate, $3–4^{mm}$ long, finely villous; stipules adnate for 8^{mm}, the free tips filiform-attenuate, about 4^{mm} long, villous; cauline leaves similar but with petioles becoming shorter as they ascend, stipules often incised and always broader than those on the radical leaves: flowers corymbose-capitate, terminating the stems, a few solitary ones or few-flowered clusters in the axils of the upper cauline leaves; hypanthium campanulate, 5^{mm} long, the bractlets linear, about as long as the subulate sepals; petals yellow, the blades broadly spatulate, 1^{mm} wide, a little longer, slightly shorter than the linear claw; stamens 15 in three rows; ovaries 5–20, glabrous, the slender styles tuberculate at base.

The type is 4405 of *Carl F. Baker's* distribution, collected by *Culbertson* July 19, 1904, at Hockett's Meadows, Tulare County, California. In the herbarium of the California Academy of Sciences are specimens of the same, collected by the author along Volcano Creek in the same region, July 17, 1903.

This species probably is most closely related to *Horkelia campestris* (Jones) Rydberg. A comparison with a duplicate of the type of the latter shows *H. mollis* to be a larger, more villous plant, the appendages of the hypanthium longer, the divisions more pointed, the petals more exserted and with blades orbicular and claws more pronounced. In general the flowers are larger.

STYRAX CALIFORNICA **fulvescens**, n. var.—Shrub a meter or so high, with stiff divaricate branches; older stems gray-black,

younger white or tawny with dense stellate tomentum: leaves orbicular-cordate, the apex obtuse or abruptly acuminate, generally slightly longer than broad, 3–6^{cm}, both surfaces stellate-tomentose, the upper less than the lower, the fulvous hairs often outlining the veins on lower surface; petioles 5–10^{mm} long: flowers 1–3, cymose, pendent, the pedicels as long as the peduncles; calyx campanulate, cuneate at base, the margin truncate but marked with 5–6 short obtuse scattered teeth, densely clothed with white or rufous tomentum; stamens 12, almost equaling the petals, attached almost the entire length of the corolla tube, filaments glabrous, ribbonlike, anthers with cell divisions white, the connective yellow, thick; style thick, broadening at base, lower half tomentose, stigma 2-lobed, surpassing the corolla.

The type of this variety was collected by the author May 17, 1904, near the Painted Cave Ranch in the Santa Inez Mountains back of Santa Barbara, California. Mr. *T. S. Brandegee* collected the same in the same mountains probably near San Marcos Pass in 1888. There is a specimen also of what seems the same collected by *J. G. Lemmon* near San Bernardino, May 1878. Near the head of Mission Creek a second collection was made by the author. This bush grew in the shade and was taller and less rufous than the others on the open hills.

This differs from the typical *S. californica* in the broader, rounder leaves, heart-shaped at base, the much denser stellate tomentum, and the general prevalence of rufous hairs especially on the calyx.

Diplacus calycinus, n. sp.—Suffrutescent, viscid-arachnoid throughout, the young stems light brown, branching diffusely: leaves elliptical to oblong, narrowed at each end, apex obtuse, base cuneate, margin revolute, entire or somewhat sinuate-denticulate, upper surface glabrous, often viscid, lower tomentose and viscid, 2–6^{cm} long, 1–2^{cm} wide; petioles very short, revolutely margined, woolly at junction: flowers axillary, the peduncles 5–7^{mm} long; lower part of fruiting calyx cylindrical, 2^{cm} long, 5^{mm} in diameter, 5-ribbed, upper half dilating abruptly to thrice the diameter of the lower, with 5 strongly keeled almost equal divisions 7^{mm} long, 3^{mm} wide at base when folded, 1^{mm} at the rounded apex, total length of calyx 3.5^{cm}; corolla light yellow, the tube curved, uniformly slender for 1.5^{cm}, dilating above, the divisions having a spread of 1.5–2^{cm}, exserted from the calyx.

This was first collected by Mr. *T. S. Brandegee* in Kaweah Canyon, Tulare County, California, July 26, 1892. The type is 4407 of *C. K. Baker's* distribution collected by *Culbertson* in the south fork of Kaweah River, 1800^{m} altitude, July 22, 1904.

This species is distinguished from allied species by the peculiar foliaceous calyx described above. The corolla in the dried specimens cannot be satisfactorily described, as in both collections the specimens are a little old.

Orthocarpus Copelandi, n. sp.—Stems about 1^{dm} high, simple or divaricately branched, minutely scabrous with short, curved hairs: lowest leaves narrowly linear-lanceolate, obtuse, 3–4^{cm} long; upper on main stem as long but twice as broad; uppermost on branches falcate, alternate or opposite: spike short and dense; lowest bracts green, the middle division like the broadest leaves, the lateral divisions spreading and very slender, about one-third as long as the middle; upper bracts shorter and broader, elliptical, rose-tipped: calyx thin and membranous, becoming globular-inflated, pink with green ribs, cleft half in front, deeper in the back, villous with short gland-tipped hairs, 7^{mm} long, 4^{mm} broad, with divisions triangular attenuate; corolla minutely glandular, 13^{mm} long, galea straight, obtuse, rose-color, ciliate, 6^{mm} long, lower lip yellow, the three sacs inflated somewhat, 5^{mm} long, middle tooth much larger than the other two: capsule bright brown, 5^{mm} long, 3.5^{mm} wide, obovate with obcordate apex, with few (apparently only two) seeds.—FIG. 1.

Collected on Mount Eddy, August 18, 1903 at an altitude of 2130^{m} by Dr. *Edwin Bingham Copeland*, in whose honor it is named. It is a beautiful species related to *O. imbricatus* and that group which contains so many closely related species. *H. E. Brown's* number 449 from the north side of Mt. Shasta is the same but very immature.

Veronica Copelandi, n. sp.—Perennial from slender, running rootstocks, about 1^{dm} high, simple, glandular-villous throughout: leaves five or six pairs, crowded on the lower part, sessile, oblong-elliptical, entire, acute, veinless, 1–1.5^{cm} long, 4–8^{mm} wide: racemes sometimes becoming 8^{cm} long, 5–15-flowered, the highest leaves often with one or two axillary flowers; bracts lanceolate, the lowest opposite, others alternate, shorter than the pedicels; peduncles 1–2^{cm} long, sometimes scarcely apparent; pedicels filiform, 5^{mm} long, a small bractlet immediately below the calyx appearing like another

FIG. 1.—*Orthocarpus Copelandi* Eastw.

sepal: sepals 4, oblong-ovate, obtuse, 3^{mm} long; corolla purple, glabrous, 9^{mm} across, the three larger divisions orbicular, entire, 4^{mm} in diameter, the smallest ovate-obtuse, 3^{mm} wide; stamens exserted, 4^{mm} long, filiform, anthers obtuse and obtusely sagittate at base, 1.5^{mm} long; stigma exserted from the opening bud, obscurely bilobed, style 7^{mm} long, filiform at base, flattening and slightly broadening towards the apex: capsules becoming almost twice as long as the calyx divisions, broadly oblong, 5^{mm} long, 3.5^{mm} wide, emarginate, the lobes and sinus obtuse; style persistent.—FIG. 2.

FIG. 2.—*Veronica Copelandi* Eastw.

This was collected on Mount Eddy at an elevation of 2500^{m} by Dr. *Edwin Bingham Copeland*, August 18, 1903, distribution of *C. E. Baker*, 1903. no. 3931. It is near to *V. Cusickii* Gray, differing in pubescence, shape of leaves and sepals, and a larger and more open-spreading corolla.

Erigeron decumbens, n. sp.—Stems several, from slender creeping rootstocks, decumbent or ascending, $1–1.5^{dm}$ high, scabrous and somewhat canescent with short appressed hairs which are glandular at base (under a lens): leaves oblanceolate to spatulate, sessile, obtuse, apparently veinless, $5–15^{mm}$ long, $3–5^{mm}$ wide, with pubescence similar to the stems: heads few, rayless, 7^{mm} high, terminating short branchlets, which are leafy near the junction with the stem and have a few scattered minute bracts on the upper part; scales of the involucre in four series, glandular-puberulent, outer ones small, reflexed-spreading, inner green-tipped, ribbed, membranous at base,

linear-lanceolate, acute, 6^{mm} long: corolla yellow, tubular, abruptly narrowed 1^{mm} above the base, the border consisting of five short, obtuse, incurved teeth; style branches exserted, the hairy tips very short: akenes slightly hairy at top; pappus simple, barbellulate, as long as the corolla.

Collected by Dr. *Edwin Bingham Copeland* on Mount Eddy, Siskiyou County, California, at an altitude of 1400^{m}, August 17, 1903. It belongs to the group which includes *E. miser* Gray, as well as many species described by Dr. E. L. Greene in *Flora Franciscana*, p. 394; but it agrees with none.

Erigeron Copelandi, n. sp.—Cespitose from an underground, branched caudex, covered with black, scale-like, imbricated bases of old petioles: radical leaves spatulate, subcanescent, with closely appressed very short pubescence; petioles equaling or longer than the blades, together $1–3^{cm}$ long, $4–8^{mm}$ wide, the petioles dilated and closely imbricated at the reddish-purple base: stems 1-flowered, $5–10^{cm}$ high, sparsely leaved with narrow linear or linear-oblanceolate leaves $5–10^{mm}$ long, becoming minute and bract-like on the glandular-puberulent upper part which is like a peduncle: heads about 6^{mm} high exclusive of the numerous, very narrow, lilac to violet rays, which are 5^{mm} long; scales of the involucre in three series, glandular-puberulent, the outermost shorter, clothed with some scattered hairs, innermost linear-attenuate, sparsely ciliate, green-ribbed, membranously margined, about 5^{mm} long; disk flowers numerous, yellow, 2.5^{mm} long, narrowed 1^{mm} above the base, glandular on the lower part, the border of five short acute incurved teeth: pappus upwardly barbellulate, simple, that of the ray flowers shorter than that of the disk, none as long as the corolla; akenes clothed with upwardly spreading hairs; stamens exserted in some flowers, pistils in others; fertile and sterile flowers in the same head, ray-flowers sterile.

Collected on Mt. Eddy, Siskiyou County, California, at an altitude of 1250^{m} by Dr. *Edwin Bingham Copeland*, in whose honor it is a pleasure to name this pretty plant. It is related to *E. pygmaeus* Greene and others of that group, but differs from all in caudex, pubescence, leaves, and heads.

Chrysopsis gracilis, n. sp.—Stems slender, simple, 3^{dm} high, loosely and sparingly villous-arachnoid, terminated by 2–4 cymose heads: leaves thin, linear-lanceolate, narrowly acuminate, $3–4^{cm}$

long, 6^{mm} wide, sessile, the upper surface somewhat dotted, lower surface arachnoid (under a lens): peduncles with pubescence like the stem but also somewhat viscid, bracts few, narrowly linear: involucral scales in about 5 ranks, the outer narrowly linear- attenuate, the others lanceolate, acute, tipped with a green and glandular spot, below yellowish, chartaceous, keeled, the innermost sometimes tinged with purple and considerably surpassing the others; heads with about 15 flowers, rayless: corolla straw color, about as long as the pappus, trumpet-shaped, gradually narrowed to the base, border with acute teeth, 1^{mm} long; style branches filiform, exserted, twining around each other at base; pappus thick, with an outer shorter row, barbellulate; akenes flat, villous, white.

Collected on Mount Eddy at an elevation of 2225^{m} by Dr. *Edwin Bingham Copeland*, August 17, 1903.

This comes very near *C. Breweri* Gray, of which it may prove to be only a variety. It differs however, in the simple instead of much branched stems, more finely arachnoid pubescence, and leaves of different outline. The involucral scales are the most distinctive; in *C. Breweri* they are attenuate and not keeled; in *C. gracilis* they are broader, acute, keeled, conspicuously green-tipped and glandular; the corollas are paler and the pappus not so rough.

Psilocarphus tenuis, n. sp.—Sparingly clothed with long loose white woolly hairs; stems filiform, erect or ascending, 3–5^{cm} high, with few slender divaricate branches: leaves oblong to elliptical, 5–10^{mm} long, 3^{mm} wide, veiny and submembranous, mucronate at apex, the base of the opposite leaves connate-clasping: heads in the forks and at the ends of the branches, the involucral leaves 4, ovate-oblong, folding over and almost concealing the flowers within, texture similar to the other leaves: fertile flowers few or many, completely enclosed by the obliquely-cuneate bracts, these gibbous, veiny, membranous, slightly woolly, 2.5^{mm} long, the apex orbicularly truncate or concave, the exserted membranous tips brownish, conspicuous, generally curved upwards: akenes shortly stipitate, narrowly obovate, 1^{mm} long: sterile flowers few, the corolla attenuate to the base, divisions reddish-brown.

Type collected at Monterey, California, by Mrs. *Joseph Clemens*, July 1905. What seems to be the same, but too young for certainty, was collected by the author at Bakersfield, Kern County, California, April 4, 1893, and at Kaweah, Tulare County, California, April 27, 1895.

This seems most distinct from all the other species in having the involucral leaves almost closing over the flowers, the peculiar concave or truncate top to the bracts enclosing the fertile flowers, and in the more veiny and membranous foliage, less woolly pubescence, and more slender habit.

Senecio Millikeni, n. sp.—Stems tall, glabrous, hollow, ribbed, paniculately branched, the slender virgate branches leafless in the lower part: leaves linear-lanceolate, narrowed at both ends, with acute apex, sessile base, margin dentate with small uneven obtuse teeth, the lower 12^{cm} long, 2.5^{cm} wide, diminishing upwards: panicle thrysiform, the peduncles and pedicels slender, bracts and bractlets attenuate, equaling or longer than the slender pedicels: heads 1^{cm} high, bracteate at base, the involucre 5^{mm} high, with glabrous scales tipped at apex with a tuft of tomentum; rays 6, 3-toothed, 7^{mm} long, style exserted 3^{mm}; disk flowers 7^{mm} long, the acute triangular teeth of the corolla slightly granular, stamens exserted but style branches surpassing them: akenes glabrous; pappus soft and abundant, about as long as the corolla.

Type collected in Natural Bridge Meadows, Tulare County, California, by *Culbertson*, Aug. 10, 1904, *C. F. Baker's* distribution 4268. It is named in honor of Mr. Culbertson's assistant.

This belongs to the polymorphous group of which *S. triangularis* was the first described. It differs from all in the narrowed bases of the leaves, the thyrsiform inflorescence, and the smaller heads.

San Francisco, California.

BRIEFER ARTICLES.

NOTES ON NORTH AMERICAN GRASSES. VI.

SYNOPSIS OF TRIPSACUM.

TRIPSACUM L., Syst., Ed. 10, 2:1261. 1759.

A GENUS of grasses confined for the most part to North America. The type species is *T. dactyloides* L.

KEY TO SPECIES.

Staminate spikelets all sessile or nearly so, outer glume coriaceous; spikes single or 2- to 3-digitate. Section DACTYLOIDES.

Blades 4 to 5^{cm} wide, pubescent on upper surface . . . *latifolium*

Blades mostly less than 2^{cm} wide

Blades 1 to 3^{mm} wide, involute . . . . . *floridanum*

Blades 1 to 2^{cm} wide, flat

Sheaths glabrous, blades glabrous except sometimes along the midrib above . . . . . *dactyloides*

Sheaths more or less hispid, or sometimes nearly glabrous, blades hispid on upper surface *dactyloides hispidum*

Staminate spikelets with one of the pair sessile, the other pedicelled, outer glume membranaceous; pistillate spikes branched, forming a fascicle. Section FASCICULATA.

Sheaths hispid . . . . . *pilosum*

Sheaths glabrous except the lowermost, or hispid only at the throat

Blades 3^{cm} or more in width, glabrous . . . . *fasciculatum*

Blades 2^{cm} or less in width, pubescent on upper surface

1.5 to 2^{cm} wide, flat or folded, culms robust . . *lanceolatum*

5 to 10^{mm} wide, more or less involute, culms slender . . . . . *Lemmoni*

Tripsacum latifolium, n. sp.—Planta robusta, vaginis glabris vel apice pubescentibus, laminis amplis, ad 4.5^{cm} latis, 70^{cm} longis, planis, supra pubescentibus subtus scabris vel glabrescentibus, spiculis sterilibus geminis sessilibus, 3–4^{mm} longis, oblongis, obtusis vel breviter acutis.

Culm robust, 1^{cm} in diameter, glabrous; sheaths glabrous or pubescent towards apex; blades ample, as much as 70^{cm} long and 4.5^{cm}

wide, pubescent above, minutely papillate-scabrous or glabrescent beneath, scabrous-ciliate on the margin; ligule very short, scarcely 3^{mm} long, fimbriate; spikes 1 to 3, similar to *T. dactyloides* but more slender, pistillate section 2 to 3^{mm} wide, staminate spikelets sessile or nearly so, 3 to 4^{mm} long, outer glume coriaceous, oblong, rounded at apex, scabrous, ciliate on marginal keels, rather minutely striate with about ten nerves.

The type specimen was collected by *H. von Tuerckheim* at Cubilquitz, Dept. Alta Verapaz, Guatemala, alt. 350^{m}, Jan. 1902, no. 8333. The only other specimen I have seen was collected by *C. Thieme* at San Pedro Sula, Dept. Santa Barbara, Honduras, alt. 500^{m}, March 1887, no. 5595^{B}. Both specimens are in the National Herbarium (Herb. John Donnell Smith).

The species is well distinguished from the other species with sessile staminate spikelets by its broad pubescent leaves.

TRIPSACUM DACTYLOIDES (L.) L., Syst., Ed. 10, **2**:1261. 1759.—*Coix dactyloides* L., Sp. Pl. **2**:972. 1753.—Usually glabrous throughout except the upper surface of the blades along the midrib near the base. This and sometimes a considerable portion of the upper surface of the blades may be sparsely pilose. The specimens from Florida and along the Gulf Coast are usually pilose in this way, or occasionally the pubescence may extend to the young sheaths of the branches. The more pubescent forms connect the species with the following subspecies, which occurs in Mexico. The terminal spikes are usually in digitate clusters of two to three, while the axillary spikes may be single. Sometimes, especially in Texas, the terminal spikes are also single (*T. dactyloides monostachyum*) (Willd.) Gray, Man. 616. 1848. *T. monostachyum* Willd., Sp. Pl. **4**:202. 1805. Type locality "Carolina meridionali."

Southern New England to Florida and Texas, mostly near the coast; but extending inland west to west Texas, and north to Nebraska, Iowa, southern Illinois, and eastern Tennessee.

If the spike is single the pistillate portion is cylindrical; if the spikes are two or three, the pistillate portions are flattened on the inner surfaces so that all together they form a cylinder, and the lower are more or less peduncled.

TRIPSACUM DACTYLOIDES **hispidum**, n. subsp.—Laminae supra hispidae; vaginae hispidae vel glabrescentes.

The staminate flowers are less chartaceous than is usual in *T. dactyloides*.

Mexico and southward. San Luis Potosi, rocky hills, Las Canoas,

Pringle 3811 (type); Jalisco, Rio Blanco, *Palmer* 509; City of Mexico, *Holway* 8; Lower California, El Taste, *Brandegee*, Nov. 1, 1902; Trinidad, Botanical Garden Herbarium 3303; Central Paraguay, *Morong* 675.

This form connects *T. dactyloides* with *T. lanceolatum.* In some specimens the upper spikelet of the staminate pair is somewhat pedicelled. *T. dactyloides* and possibly some of the other species may occur widely distributed in South America. Information on this point is desired.

TRIPSACUM FLORIDANUM Porter, Contr. Nat. Herb. **3**:6. 1892. PORTER'S herbarium name was published by Dr. VASEY in his monograph of the grasses of North America. Type locality "Florida (*A. P. Garber*) and Texas (*G. C. Nealley*);" duplicate type in National Herbarium. *T. dactyloides floridanum* Beal, Grasses **2**:19. 1896. There are no specimens of this species from Texas in the National Herbarium, nor are there any so labeled by Dr. VASEY; consequently the Texas locality given above is uncertain and is probably incorrect.

Our specimens are all from the vicinity of Miami, Florida, *Garber* 454, June 1877 (type); *Pollard & Collins* 272, April 1898; *Eaton* 530, Dec. 1903; *Hitchcock*, March 1903.

Distinguished from *T. dactyloides* by its smaller size and much narrower leaves.

TRIPSACUM FASCICULATUM Trin.; Ascherson, Bot. Zeit. **35**:521. 1877.—Well distinguished by its ample glabrous leaves, which are as much as 6.5^{cm} wide and 70^{cm} long, resembling leaves of Indian corn (*Zea mays* L.). Plant glabrous throughout; spikes branched, forming a fascicle; staminate portion slender and more or less flexuous, the spikelets 5 to 6 ^{mm} long and broadest near the top.

The name first appears in the second edition of STEUDEL'S Nomenclator, **2**:712, as *Tripsacum* "fasciculatum Trin. Mpt. Mexico. *T. dactyloides* Schlecht. in Linnaea VI." The latter name is a *nomen nudum*, as is also *T. fasciculatum* Trin. in Steud. Gram. **1**:363, and in Ruprecht, Bull. Acad. Brux. **9**:243. The first description appears to be by ASCHERSON[1] in 1877, Bot. Zeit. **35**:525, where a specimen from "Pr. Hacienda de la Laguna (Schiede)" is designated as the type. FOURNIER, Mex. Gram. 69. 1881, includes the name without description and cites the following specimens: Hacienda de la Laguna (*Schiede* 947); Orizaba

[1] ASCHERSON had previously mentioned the species and given a brief description as follows: "Diese Art besitzt Blätter von der Breite der Maisblätter, und die zahlreichen, schlaffen, männlichen Inflorescenzzweige, deren Aehrchen kleiner als bei *T. dactyloides* sind, erinnern ebenfalls an *Euchlaena*." (Verh. bot. Ver. Pr. Brandenb. **17**:79. 1875, in a footnote to an article on *Euchlaena mexicana*.)

(*Bourgeau* 3138); Mirador (*Liebmann* 549); Zacuapan pr. Jalapa (*Galeotti* 5796); Arumbaro (*Galeotti* 5844). The BOURGEAU and LIEBMANN specimens are in the National Herbarium; also *Brade* 16174, from Costa Rica.

TRIPSACUM LANCEOLATUM Rupr.; Fournier, Mex. Gram. 68. 1881.—Leaves mostly 1 to 2^{cm} broad, pubescent on the upper surface; staminate flowers 7 to 9^{mm} long, spindle-shaped, often rather abruptly narrowed above the middle.

Mexico. Sonora, Guadaloupe Cañon, International Boundary Commission, 2035; Durango, *Palmer* 537; Oajaca, Villa alta, *Liebmann* 547; Lower California, Sierra de San Francisquito, *Brandegee* 6, Sept. 1899; Jalisco, between Huejuquilla and Mesquitec, *Rose* 3570. In addition to these specimens in the National Herbarium, FOURNIER gives the following: Inter Victoria et Rio Blanco (*Karwinsky*); Borrego prope Orizaba (*Botteri* 1213 in herb. VAN HEURCK); Mirador (*Schaffner*); Tacubaya (*Schaffner* 41 in herb. FRANQUEVILLE); Secus Amnem in herbosis pr. Pedregal (*Bourgeau* 444); Aguas Calientes (*Hartweg* 252). *Liebmann* 547 is also cited by FOURNIER and it is upon this specimen that I have based my identification of the species. FOURNIER's description does not apply in all respects to the plants which I have included under this species. He states that the culms are pilose, which is not true of any of the specimens I have seen. Neither are both staminate spikelets pedicelled, as he describes.

The name first appears in Plant. Hartw. Addenda, p. 347. In the body of the work (p. 28) no. 252 is listed without description as *T. dactyloides* "in saxosis, Aguas Calientes." In the addenda this is corrected as follows: "n. 252 est species a Tripsaco dactyloide distincta, T. lanceolata, Ruppr. ex cl. Rupprecht in Litt." FOURNIER (*l. c.*) cites *T. lanceolatum* Rupr. in Benth. Pl. Hartw. 247. Under the circumstances I think HARTWEG'S no. 252 from Aguas Calientes should be considered as the type of *T. lanceolatum* rather than KARWINSKY's specimen, the first cited by FOURNIER.

FOURNIER cites as a synonym of this "*T. acutiflorum* Rupr. mss. in herb. Petrop." Under the rules of the recent code *T. acutiflorum* was not published. FOURNIER (*l. c.* 69) also mentions without description, var. *β monostachyum* from San Luis Potosi (*Virta* 1447). I have not seen this specimen.

TRIPSACUM PILOSUM Scribn. & Merr., Div. Agrost. Bull. 24:6. 1901. —Type locality Mexico. "Collected on the road between Colotlan and

Bolaños, State of Jalisco, 2841 *J. N. Rose*, September 7, 1897." Specimen in National Herbarium.

The preceding species, together with this and the following, form a rather closely connected series. The type of *T. pilosum* is distinguished by the strongly papillate-hirsute sheaths, and the blades pubescent upon both surfaces, but these characters are much less marked in some of the specimens which agree with the type in other particulars.

I have referred here the following specimens: Jalisco, Rio Blanco, *Palmer* 508; Cañon near Guadalajara, *Pringle* 2623, and hills near Guadalajara, *Pringle* 2611; San Luis Potosi, limestone ledges, Tinamel, *Pringle* 3993; and San Jose Pass, *Pringle* 3447.

TRIPSACUM LEMMONI Vasey, Contr. Nat. Herb. **3**:6. 1892. Type locality, "Huachuca Mountains, Arizona (*J. G. Lemmon*)." Type specimen in National Herbarium. *T. dactyloides Lemmoni* (Vasey) Beal Grasses **2**:19. 1896.

Plant glabrous throughout except the lowermost sheaths, which are more or less hispid. The leaves are long and narrow, 5 to 10^{mm} wide, and in herbarium specimens inrolled at the margins.

In addition to the type specimen I have included two Mexican specimens, Jaral, Gebirgsthäler, *Schumann* 1718, and Jalisco, Mountains near Guadalajara, *Pringle* 2610. These two specimens have the spikes digitate instead of fascicled as in Arizona specimen, but the latter has the lateral spikes in ones or twos.—A. S. HITCHCOCK, *U. S. Dept. Agric., Washington, D. C.*

CURRENT LITERATURE.

BOOK REVIEWS.

Vegetable foods.

THIS well-known work of MOELLER on this subject,[1] first put out about twenty years ago, has played an important part in connection with the increasing use of the microscope as a practical instrument for recognizing vegetable substances in a more or less finely divided state. Many changes have taken place within this period, not only in the art of substitution and adulteration, which is as alert and progressive as any that might be mentioned, but also in matters of legitimate commerce. Hence a thoroughgoing reworking of the field has been made necessary. In connection with this service, Prof. A. L. WINTON, now of the Connecticut Agricultural Experiment Station, a pupil of MOELLER, has furnished very important aid in the form of excellent figures as well as text.

The scope of the work is in general indicated by the title and those articles here treated are with few exceptions used as food for man or beast, the term food being defined so as to include such articles as flavoring agents as well as tea, coffee, and cacao. Under the appropriate headings those substances are also described and figured which occur as impurities, substitutions, and adulterations. Since it often happens that condiments are also official drugs, many chapters have a strong pharmaceutical interest. A few articles are considered which have their chief significance as drug products, e. g., sandal wood, guarana, cubebs, cola, salep, and calamus.

In the treatment of the individual articles, the book is distinguished by a concise and exact statement of the features, gross and microscopic, characterizing the structures concerned, dimensions frequently cited giving definiteness to terms of size.

As valuable as the excellent text, are the numerous drawings illustrative of it. A large number are original, many being by Dr. WINTON. A bibliography of the most important articles written on each subject closes the consideration. One novel feature among the illustrations is seen in the gross pictures of the leaves discussed. Here a direct print is made on a sensitive surface, using the leaf itself as an opaque object. This method has been successfully used before by a number of authors with various objects and here the result is in general successful. Frequently a very considerable amount of detail has

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[1] MOELLER, JOSEF, Mikroskopie der Nahrungs- und Genussmittel aus dem Pflanzenreiche. Zweite gänzlich umgearbeitete und unter Mitwirkung A. L. Winton's vermehrte Auflage. 8vo. pp. xvi+599. *figs.* 599. Berlin: Julius Springer. 1905. *M* 18; geb. *M* 20.

been obtained in the half tone reproductions of these so-called "autophotograms."

The revision of this important work has again brought it to the front and promises to continue it as one of the valuable literary aids to the investigator of pure foods.—RODNEY H. TRUE.

ALMOST simultaneously with the foregoing has appeared in this country a similar compendium by the same team; this time the pupil leads and the master is the collaborator.[2] The general plan and purpose of Dr. WINTON'S weighty volume are similar to those of Dr. MOELLER'S. The fact that it is in English will give it a sale that the German book could not hope to attain among the food commissioners and inspectors and the official chemists, to whom at present such a work makes its chief appeal. By reason of the existing agitation in this country on the subject of pure foods and drugs, the enforcement of existing laws, and the imminence of new and more exacting legislation, this publication is peculiarly timely. The botanical features are on the whole reasonably accurate, especially the anatomy, which is most fundamental. The definitions in the glossary are not always above criticism, and accuracy would not have rendered them less practical. The illustrations are numerous and good, particularly the original ones. A strong foreign flavor pervades the whole book, showing the impress of the work of MOELLER, yet curiously his second edition is not cited in the bibliography. The arrangement of material, analytic keys, lists of adulterants, and the suggestions as to diagnosis are sure to be of great practical service in the new campaign against sophistication by unscrupulous manufacturers and dealers.—C. R. B.

MINOR NOTICES.

Cryptogamic flora of Brandenburg.[3]—This monumental work begins its seventh volume with the first fascicle of the Ascomycetes. Its character and scope are so well known that the announcement of its publication and contents will suffice to secure the orders of all who concern themselves with this group. Hemiasci are treated by G. LINDAU; Saccharomycetineae by P. LINDNER; Protascineae by G. LINDAU; Exoascaceae, Erysiphaceae, Perisporiaceae, Macrothyriaceae, and Aspergillaceae by F. NEGER; Onygenaceae, Elaphomycetaceae, Terfeziaceae, and Tuberaceae by P. HENNINGS.—C. R. B.

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[2] WINTON, A. L., The microscopy of vegetable foods, with special reference to the detection of adulteration and the diagnosis of mixtures. With the collaboration of Dr. JOSEF MOELLER. Imp. 8vo. pp. xvi+701. *figs.* 589. New York: John Wiley & Sons. 1906. $7.50.

[3] Kryptogamenflora der Mark Brandenburg, Band 7, Heft 1. Pilze. Von P. HENNINGS, G. LINDAU, P. LINDNER, F. NEGER. 8vo. pp. 160. *figs.* 17. *pls.* 8. Leipzig: Gebrüder Borntraeger. 1905. *M* 1.50. (Not sold separately.)

NOTES FOR STUDENTS.

What is a species?—The many discussions as to what is a species have resulted in a general appreciation of the facts that species are not all of equal rank, that they are distinguished by more or less arbitrary characters, and that although many species are real natural groups of individuals, many others are simply arbitrary groups, associated for the sake of convenience. After reviewing the various methods of distinguishing species, KUPFFER[4] concludes that no method will apply in all cases, that all methods are of importance, and that when the several methods are used conjointly, little difficulty is experienced.

KUPFFER then turns to the methods of KÖLREUTER, based upon the sterility of hybrids, as a method which has not been used to the extent its merits warrant. Sterility of the hybrids being presumably due to defective germ-cells, he depends for his measure of sterility upon the condition of the pollen, basing his method upon the fact pointed out a few years ago by JENCIC[5] that viable pollen swells immediately upon the introduction of water, while the sterile pollen remains shrunken, and that this capacity of the normal pollen to swell is retained for many years in herbarium materials (more than 50 years in Viola, *fide* KUPFFER).

Although the author recognizes that considerable sterility of the pollen has been observed in many "good" species, he has himself never found a pure species in which more than a few (ein Paar) per cent. of the pollen grains remained shrunken, the implication being that the reported instances would bear further consideration.

After examining a number of species and their hybrids, especially among the Violaceae, he concludes that when a supposed hybrid shows much less fertility of the pollen than its supposed parents, it is not a *necessary* but a *sufficient* proof (1) that the supposed hybrid is truly a hybrid, and (2) that its parents belong to distinct species.

Application of this method is then made with interesting results to forms of Potentilla, Viola, Thymus, etc., which have puzzled the systematist.—GEORGE H. SHULL.

Propagation of grain rust.—Further comments by Dr. JAKOB ERIKSSON[6] on the question of the origin and distribution of the rust-diseases of plants have recently been presented to the botanical public through separata. The author has not essayed so much to put forth new facts, as to bring together and review those recently published, in so far as they bear upon his mycoplasm theory, giving especial attention to adverse criticisms.

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[4] KUPFFER, K. R., Kölreuters Methode der Art Abgrenzung nebst Beispielen ihrer Anwendung und einigen allgemeinen Betrachtungen über legitime and hybride Pflanzenformen. Acta Hort. Bot. Univ. Imp. Jurjevensis **6**: 1–19. 1905.

[5] JENCIC, Untersuchungen des Pollens hybrider Pflanzen. Oesterr. Bot. Zeits. **50**: 1, 41, 81. 1900.

[6] ERIKSSON, J. Zur Frage der Entstehung und Verbreitung der Rostkrankheiten der Pflanzen. Arkiv för Botanik **5**[3]: 1–54. 1905.

He maintains that after taking into consideration the studies and observations of MARSHALL WARD and PLOWRIGHT in England; MCALPINE and COBB in Australia; BOLLEY, HITCHCOCK, and CARLETON in North America; BARCLAY in India; KLEBAHN, DIETEL, SCHROETER, and MAGNUS in Germany; LAGERHEIM in Sweden, and others, the wintering of the uredo-bearing mycelium, or of the uredospores, so as to be a source of infection for the coming season, has not been proven. The evidence, chiefly as brought forward by KLEBAHN, to show that the first appearance of the rust in spring can often be accounted for by uredospores being carried long distances by the wind, is reviewed, and the conclusion reached that this is an assumption based on no direct evidence and highly improbable.

The author then enters upon the vital part of the subject and discusses the mycoplasm theory and its recent criticism, especially that which has been most ably presented by KLEBAHN and MARSHALL WARD. After an extended examination of the works of these authors, he finds that his theory has not been affected. He directs attention to a report by BIFFEN of recent experiments in hybridizing wheat carried on at Cambridge, England, in which the appearance of rust on the plants can best be explained by assuming that the mycoplasm of certain varieties was transmitted through the pollen to the resulting hybrid. —J. C. ARTHUR.

Gynodioecism.—CORRENS[7] has presented a second[8] report on the gynodioecism of *Satureia hortensis* and *Silene inflata*, giving full confirmation of his earlier conclusion that the pistillate form produces only, or mostly, pistillate offspring when fertilized, as it must be, by the bisporangiate form. If the pistillate form is a mutant from the bisporangiate and differs from the latter by the possession of a distinct hereditary unit, as suggested by Burck,[9] all the seeds produced by a pistillate plant are of hybrid origin, and the observed facts would be best explained as a case of dominance of the newly risen character over the older. In Satureia this dominance (?) is complete, but in Silene the offspring of the pistillate plants were pistillate in only 87–93 per cent., the rest being bisporangiate. Although this behavior looks very much like Mendelian inheritance, a number of cases are cited in which quite contradictory results have been obtained, so that while the author states it as a law that each sex has a *tendency* to transmit its own sex form, he does not look upon this as dominance in the Mendelian sense.—GEORGE H. SHULL.

An ear of corn.—The origin of such economic plants as wheat and maize, which have a wide distribution in cultivation but are unknown in the native

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[7] See BOT. GAZETTE **39**:304. Ap. 1905.

[8] CORRENS, C., Weitere Untersuchungen über die Gynodioecie. Ber. Deutsch. Bot. Gesell. **23**:452–463. 1905.

[9] BURCK, W., Die Mutation als Ursache der Kleistogamie. Recueil Trav. bot. Néerl. **1–2**:95 *sqq*. 1905.

state will doubtless always be an interesting subject for speculation. The most satisfactory hypothesis for the origin of maize, and that which has been until this time rather generally accepted, derives it from the teosinte (Euchlaena). It has been thought that the ear was formed by an abnormal coalescence of the pistillate spikes of that plant. The ease with which maize and teosinte may be crossed gives strong support to the theory that they are nearly related.

An altogether different view of the origin of the pistillate spike of maize is presented by MONTGOMERY[10] and much evidence is given in its support. His hypotheses are that the ear of corn is the homologue of the central spike of the staminate inflorescence; and that the progenitor of maize was a much branched plant, bearing only terminal branched inflorescences of bisporangiate flowers. The chief support of these hypotheses is derived from abnormal development of pistillate and bisporangiate flowers in the staminate inflorescence, and *vice versa*. A number of photographs show these abnormalities and *fig. 14* represents a plant, denuded of its leaves, showing that the same number of internodes intervene between the central axis and the ear as are found between the ear and the tassel. Nothing in this new interpretation of the pistillate spike of maize need lessen the conviction of its near relationship with Euchlaena.—GEORGE H. SHULL.

The laws of inheritance.—CORRENS[11] has published a lecture on the laws of inheritance which presents in a very satisfactory manner the recent advances which have been made in this discipline. He would include in hybridization every union between two germ-cells having one or more different character-units. The laws of dominance and of the purity of the parental gametes are illustrated from his own experiments on Urtica, Mirabilis, and Zea, and emphasis is given to the fact that these two laws are absolutely unrelated to each other, and that reference to them jointly as Mendel's Law is misleading.

Latency is considered at some length, but the present state of knowledge of this subject leaves much to be desired. He makes a proper distinction between latency in the sense of invisibility, and *true* latency in which there is actual inactivity of a unit that may be changed at times from a passive to an active state.

Regarding the relation between MENDEL'S and GALTON'S laws, he holds with DARBISHIRE,[12] that both are correct and the antagonism only apparent, due to the different manipulation of the data.

CORRENS still maintains that sex is fundamentally unlike the unit-characters which behave in accord with MENDEL'S laws. Touching on xenia and tel-

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[10] MONTGOMERY, E. G., What is an ear of corn? Popular Sci. Monthly **68**: 55–62. *figs. 14*, Jan. 1906.

[11] CORRENS, C., Ueber Vererbungsgesetze. 8vo. pp. 43. *figs. 4*. Berlin: Gebr. Borntraeger. 1905.

[12] DARBISHIRE, A. D., On the supposed antagonism of Mendelian to biometric theories of heredity. Mem. and Proc. Manchester Lit. and Philos. Soc. **49**. no. 6. 1905. 19 pp.

egony, he holds that neither exists in the strict sense, namely that ids may escape from the germ-cells to produce modification in the surrounding maternal tissues, or to be transferred thence into subsequent germ-cells.—GEORGE H. SHULL.

Heterostyly in Primula.—The inheritance of heterostylism in Primula has been investigated by BATESON and GREGORY,[13] who find that there is general agreement with Mendelian expectation, the short style being dominant over the long style. A second character, a yellow flush in the center of the flower, which was found associated with an "equal-styled" condition, also proved to be Mendelian and capable of being transferred by crossing to the short-styled form. The investigation showed that whenever the yellow flush occurs in a combination in which the long style would be expected, the styles do not develop beyond the level of the anthers, thus forming the "equal-styled" type. Several aberrant results were observed, the most noteworthy being a case in which a single plant indicated a different composition of its germ-cells, according as it was used as the pollen-parent or pistil-parent.—GEORGE H. SHULL.

Asparagus rust.—SMITH[14] has published a final account of his investigation of the asparagus rust in California. One of the most important results of his work is the demonstration of the fact that the spores of this rust depend upon dew for the moisture required for germination. The more detailed account of the water relation of this rust was published in this journal.[15] This discovery suggested certain practical methods of controlling the rust, such as planting the rows with the wind and preventing weeds and other plants or trees from forming a windbreak close about the asparagus field. In other words, the field should be well ventilated. The bulletin will long continue to be the standard work of reference for information upon the subject.—E. MEAD WILCOX.

Potato scab.—HENDERSON[16] has recently published the results of his studies of the methods of control of the potato scab. He found that rolling the potato tubers in sulfur did not prevent the scab, and this is in accord with results secured by other investigators. Formalin and corrosive sublimate gave equally good results with the factor of safety in use in favor of the formalin. If treated potatoes were planted in soil in which "scabby" potatoes had grown the previous season, the scab appeared in spite of the treatment. This emphasizes the necessity of preventing new ground from becoming infected with the disease by planting none but healthy tubers.—E. MEAD WILCOX.

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[13] BATESON, E., and GREGORY, R. P., On the inheritance of heterostylism in Primula. Proc. Roy. Soc. London B. **76**:581–586. 1905.

[14] SMITH, R. E.—Asparagus and asparagus rust in California. Bull. Calif. Exp. Stat. 165:1–99. *figs. 1–46*. 1905.

[15] SMITH, R. E., BOT. GAZETTE **38**:19–43. *figs. 1–21*. 1904.

[16] HENDERSON, L. F., Potato scab. Bull. Idaho Exp. Stat. 52:1–8. 1906.

Nuclear division in Ascomycetes.—GUILLIERMOND[17] has continued his studies on nuclear division in the Ascomycetes, which support in all essentials the conclusions of HARPER and contravene those of MAIRE (except as to Galactinia), though they are perhaps not irreconcilable with them. However, his descriptions are not so detailed as those of HARPER in his last paper on Phyllactinia, especially as it relates to the centers of spindle formation. In this paper GUILLIERMOND discusses chiefly the mother-cells of the asci and secretion. The species studied comprise *Pustularia vesiculosa, Aleuria cerea, Peziza rutilans, P. Catinus,* and *Galactinia succosa.* —B. M. DAVIS.

Soil waters.—CAMERON and BELL show[18] that as a rule the various mineral constituents of the soil solutions exist in sufficient concentration for the growth of crops, and that the magnitude of the concentrations is *practically the same for all soils*, because, generally speaking, soils contain all the common rock forming minerals, some of each species presenting its surfaces to the solvent action of the soil water; and on account of hydrolysis of the solutes this solvent action is continuous. The paper strongly supports the previous work of the Bureau of Soils which has been so much criticised, often on *a priori* grounds.—C. R. B.

Non-infection by rusts.—*Erysiphe graminis* has a number of biologic forms which are confined to special hosts. Thus conidia from the form on wheat will not infect barley and that on oats will not infect wheat. SALMON[19] has recently shown that the reason of the non-infection is not due to inability on the part of the conidia to germinate, but because the haustoria cannot establish relations with the cells of the host plant.—B. M. DAVIS.

Endoparasitic adaptation.—SALMON[20] shows that *Erysiphe graminis* adapts itself readily to an endophytic life. When spores are sown on a wound in oats or barley the mycelium ramifies in the intercellular spaces and haustoria are abundantly produced. Conidiophores develop profusely and perfect conidia where they arise on a free surface; and they even break through a weak barrier when they develop in intercellular spaces.—C. R. B.

Greening of seeds.—ERNST[21] finds that during the ripening of the fruit of *Eriobotrya japonica* the seeds become green, quite independent of light, by reason of the greening of the amyloplasts. The process begins at the plumule of the

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[17] GUILLIERMOND, A., Remarques sur la karyokinèse des Ascomycètes. Ann. Mycol. **3**:343–361. *pls. 10–12.* 1905.

[18] CAMERON, F. K., and BELL, J. M., The mineral constituents of soils. U. S. Dept. Agric., Bur. Soils Bull. 30. pp. 70.

[19] SALMON, E. S., On the stages of development reached by certain biologic forms of Erysiphe in cases of non-infection. New Phytol. **4**:217. 1905. *pl. 5.*

[20] SALMON, E. S., On endophytic adaptation shown by *Erysiphe graminis* DC. under cultural conditions. Phil. Trans. Roy. Soc. London B. **198**:87–97. *pl. 6.* 1905.

[21] ERNST, A., Das Ergrünen der Samen von *Eriobotrya japonica.* Beihefte Bot. Centralbl. **19**[1]: 118–130. *pl. 2.* 1905.

embryo and progresses from this region to the inner and outer faces of the cotyledons. Complete greening, however, only follows illumination.—C. R. B.

The nucleus and secretion.—In the nectar glands on the stipules of the *Vicia Faba*, according to STOCKARD,[22] the nucleus does not give out granular material directly to the cytoplasm, but it transmits a substance which results in the formation of granules. Changes which occur in the cytoplasm during secretion seem to be controlled by the nucleus.—CHARLES J. CHAMBERLAIN.

Black rot of cabbage.—HARDING, STEWART, and PRUCHA[23] find much of the cabbage seed in the market contaminated with *Pseudomonas campestris*, which may survive and become a source of infection to seedlings. They advise sterilizing seed by soaking for fifteen minutes in $HgCl_2$ 1:1000, or in formalin 1:240.—C. R. B.

Movement of diatoms, etc.—JACKSON suggests[24] that the evolution of oxygen is the true cause of movements of diatoms, desmids, oscillaria, nostoc, etc. He has been able to imitate the movements by those compressed tablets and bits of aluminum of proper shapes which evolve gas.—C. R. B.

Anatomy of Claytonia.—A study of this genus by THEO. HOLM forms one of the Memoirs of the National Academy,[25] where it may be overlooked by botanists. It contains some of the accumulating details which a master hand must some day correlate.—C. R. B.

Apothecia of lichens.—GERTR. P. WOLFF[26] through some studies on the development of the apothecia in a number of lichens argues against LINDAU'S terebrator theory of the function of the trichogynes in lichens.—B. M. DAVIS.

Intercellular ducts.—The intercellular spaces in the cotyledons of Leguminosae function at the beginning of germination as conducting canals for aleurone which becomes dissolved and diffuses through them.[27]—C. R. B.

Mustiness.—The peculiar musty odor acquired by damp straw or corn is due, according to ROUSSEU,[28] to the oospora form of *Streptothrix Dassonvillei* and not to other of the fungus flora found thereon.—C. R. B.

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[22]STOCKARD, CHAS. R., The structure and cytological changes accompanying secretion in the nectar glands of *Vicia Faba*. Science **21**:204–5. 1906.

[23]HARDING, H. A., STEWART, F. C., PRUCHA, M. J., Vitality of the cabbage black rot germ on cabbage seed. N. Y. Agr. Exp. Sta. Bull. 251: 177–194. 1905.

[24]JACKSON, D. D., Movements of diatoms and other microscopic plants. Jour. Roy. Mic. Soc. **1905**: 554–7.

[25]HOLM, THEO., Claytonia, a morphological and anatomical study. Mem. Nat. Acad. Sci. **10**: 27–37. *pl. 1, 2.* 1905.

[26]WOLFF, GERTR. P., Beiträge zur Entwicklungsgeschichte der Flechtenapothecien. Flora **95**:31. 1905.

[27]JOFFRIN, H., Rôle circulatoire des méats intercellulaires dans les cotylédons des Légumineuses au début de la germination. Rev. Gén. Bot. **17**: 421–2. 1905.

[28]BROCQ-ROUSSEU, Contributions a l'étude des causes qui provoquent l'odeur de moisi des grains et fourrages. Rev. Gén. Bot. **17**: 417–420. 1905.

NEWS.

PROFESSOR J. C. ARTHUR spent the greater part of January at the New York Botanical Garden in a study of Uredineae.

PROFESSOR B. M. DUGGAR has been spending the winter in research at the Botanical Institute at Montpellier, directed by Professor CH. FLAHAULT.

THE *Bulletin* de l'Académie Internationale de Géographie Botanique an nounces the limitation of leading articles to thirty-two pages. We hope the movement will become general among journals. By proper condensation an author can say all he is entitled to say on one subject in such a space.

DR. JESSE M. GREENMAN spent some six weeks in Yucatan and adjacent Mexico collecting plants for the Field Natural History Museum, of whose herbarium he is assistant curator. He had a violent attack of malarial fever which interfered seriously with his work, but he has returned in good health and with fair collections.

LAST SUMMER after leaving the Vienna Congress, Professor GEORGE F. ATKINSON spent some time in the vicinity of Nice, Paris, and especially in the Jura mountains in the vicinity of Pontarlier, studying the fleshy fungi. He collected over 300 species, made photographic studies, and preserved material for morphological investigation.

THE VIENNA CONGRESS nominated as presidents of the Committee of Organization for the Brussels Congress of 1910 LÉO ERRERA and TH. DURAND. On account of the lamented death of Professor ERRERA the *Association internationale des botanistes* has named Senator Count OSW. DE KERCHOVE DE DEUTERGHEM as his successor. M. ÉMILE DE WILDEMAN has been made general secretary.

MR. J. B. ELLIS, whose taxonomic work on North American fungi is known the world over through his numerous publications and the important sets of *exsiccati* issued by him and Mr. EVERHARDT, died at his home in Newfield, New Jersey, December 30, 1905. A biographical sketch of Mr. ELLIS was published in this journal in November 1890. His herbarium and library have been for some years the property of the New York Botanical Garden.

PROFESSOR WILLIAM WHITMAN BAILEY will retire from the faculty of Brown University at the close of the present academic year. He has been connected with the University for nearly twenty-eight years, twenty-five of them as professor of botany. For some years he has been suffering from ill health and feels it wise not to carry longer the burden of regular classroom work. Yet he will retain close connection with the University, and advise in many of its affairs.

A NEW JOURNAL, *Le Bambou*, has been established by JEAN HOUZEAU DE LEHAIE, Ermitage, Mons, Belgium. The English part of the trilingual prospectus contains some interesting information for our readers, and is at the same time so amusing in its construction and spelling that we reprint part of it.

"Our aim is the facility for botanists and lovers of Bamboo of communicating their studies and desiderata, and exchanging their observations. We claim likewise as design to let better know the horticultural value of these plants and, giving information on the process of culture and on the places from where they kan be obtained, to spread as much as possible their use in parks and gardens. Each number shall contain: 1th a technical part, 2d a practical part.

"The technical part for wohm we can rely on the cooperation of distinghuished botanists wohm names are know from a long time, shall comprise the description of new or little known species, with plates, or cuts, the critical examination of the nomenclature and synonymy and the bibliography.

"These studies shall compose an ensemble which will become a vade mecum necessary to all lovers of Bambusaceae who whish to make serious study. . . .

"Briefly, the classification of Bamboo is still on many points somewhat wavering and the principal design of the technical part is to cooperate to its perfection.

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"We hope that our correspondents will be so good as to communicate the tittles of the books on this matter within their knolege.

"The practical part shall contain such advices of culture our essais, began since 1883, entitle us to give; . . . .

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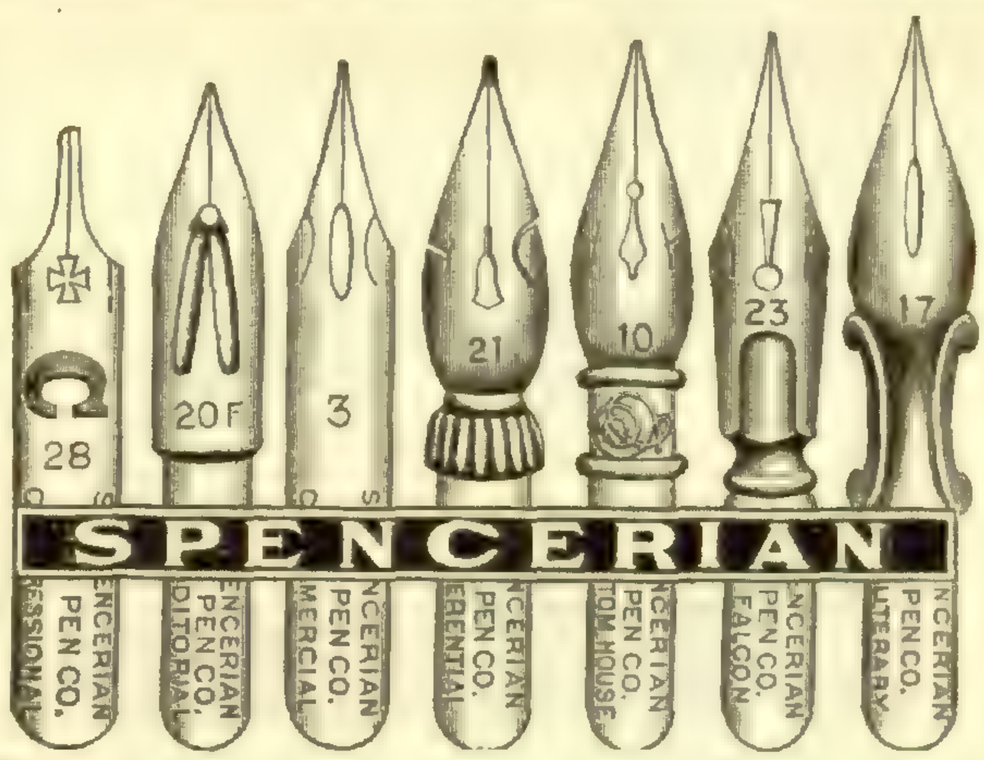

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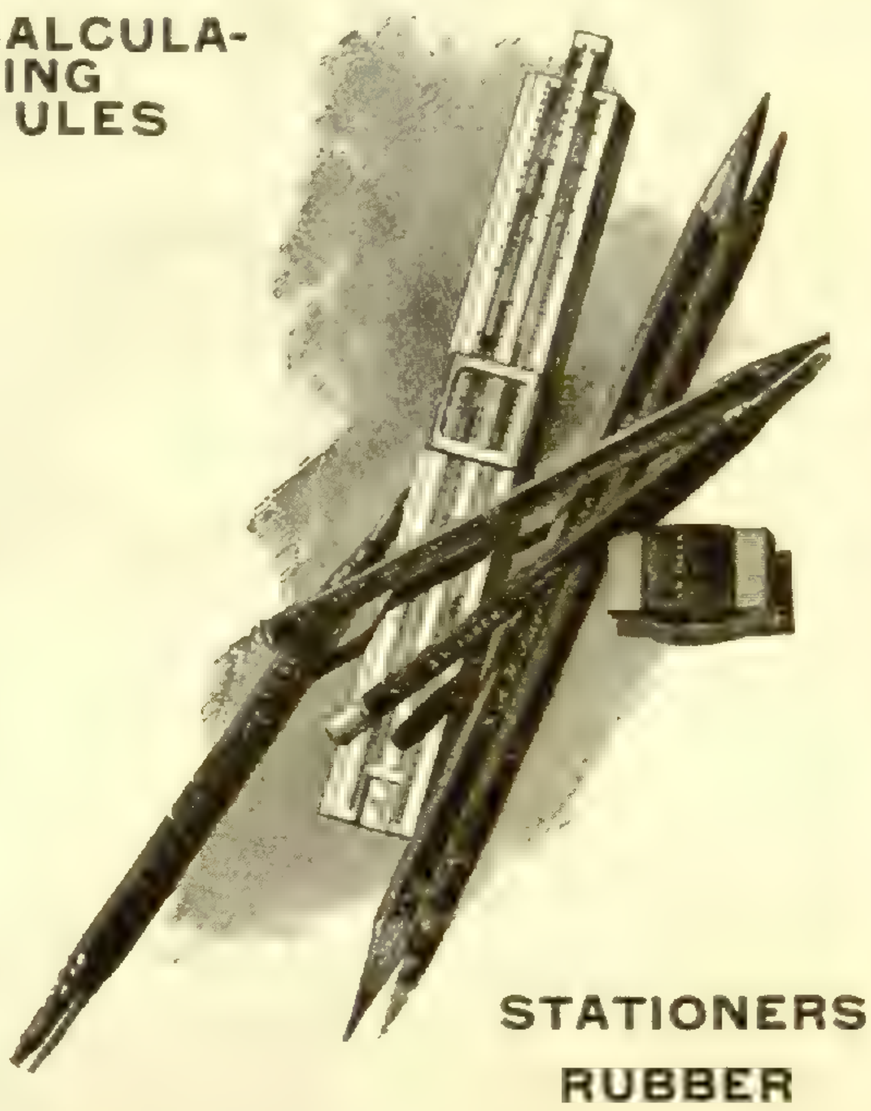

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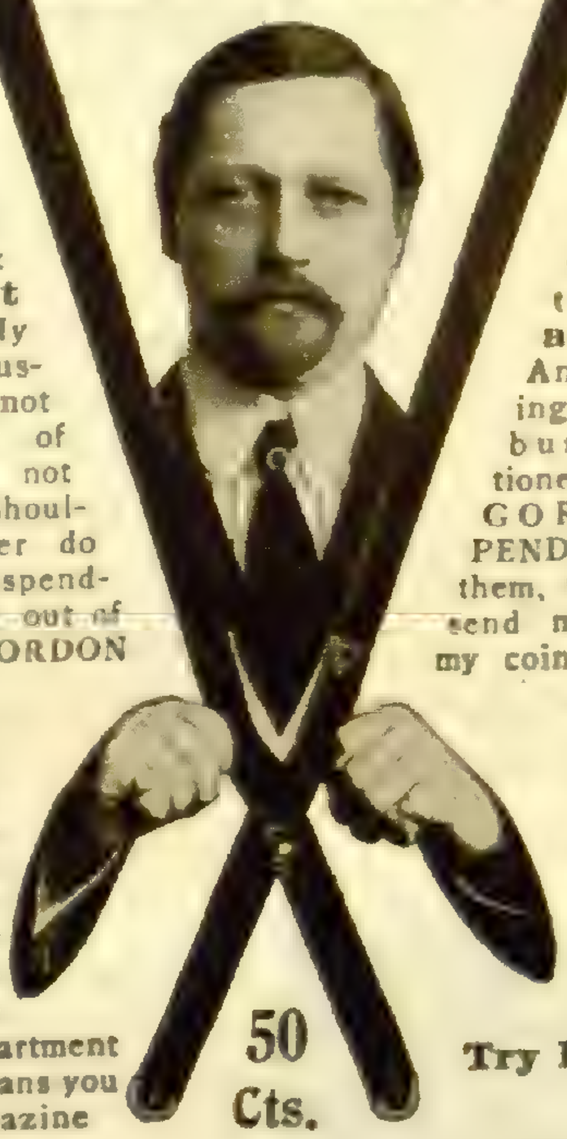

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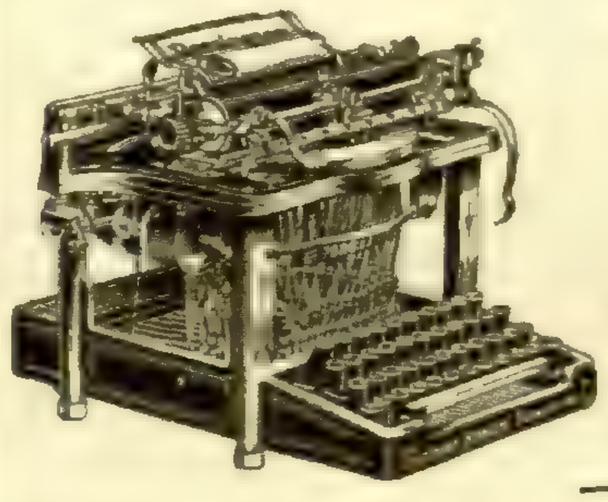

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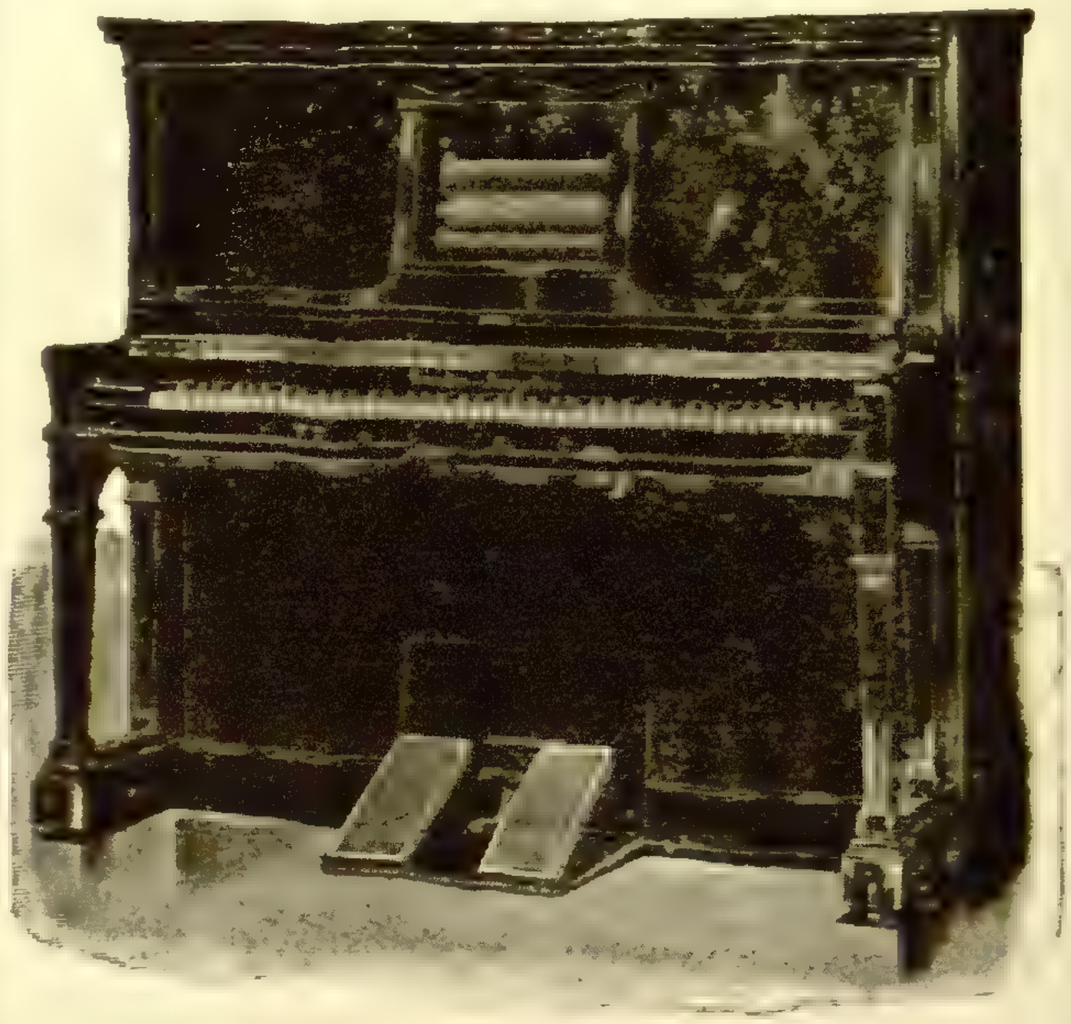

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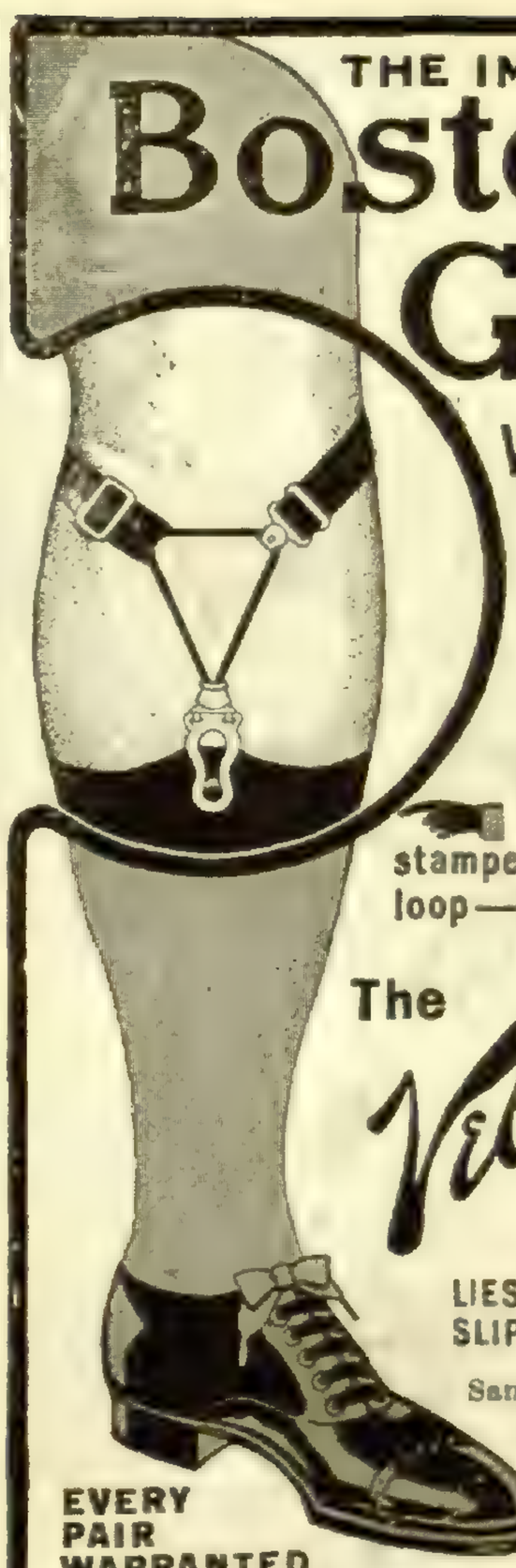

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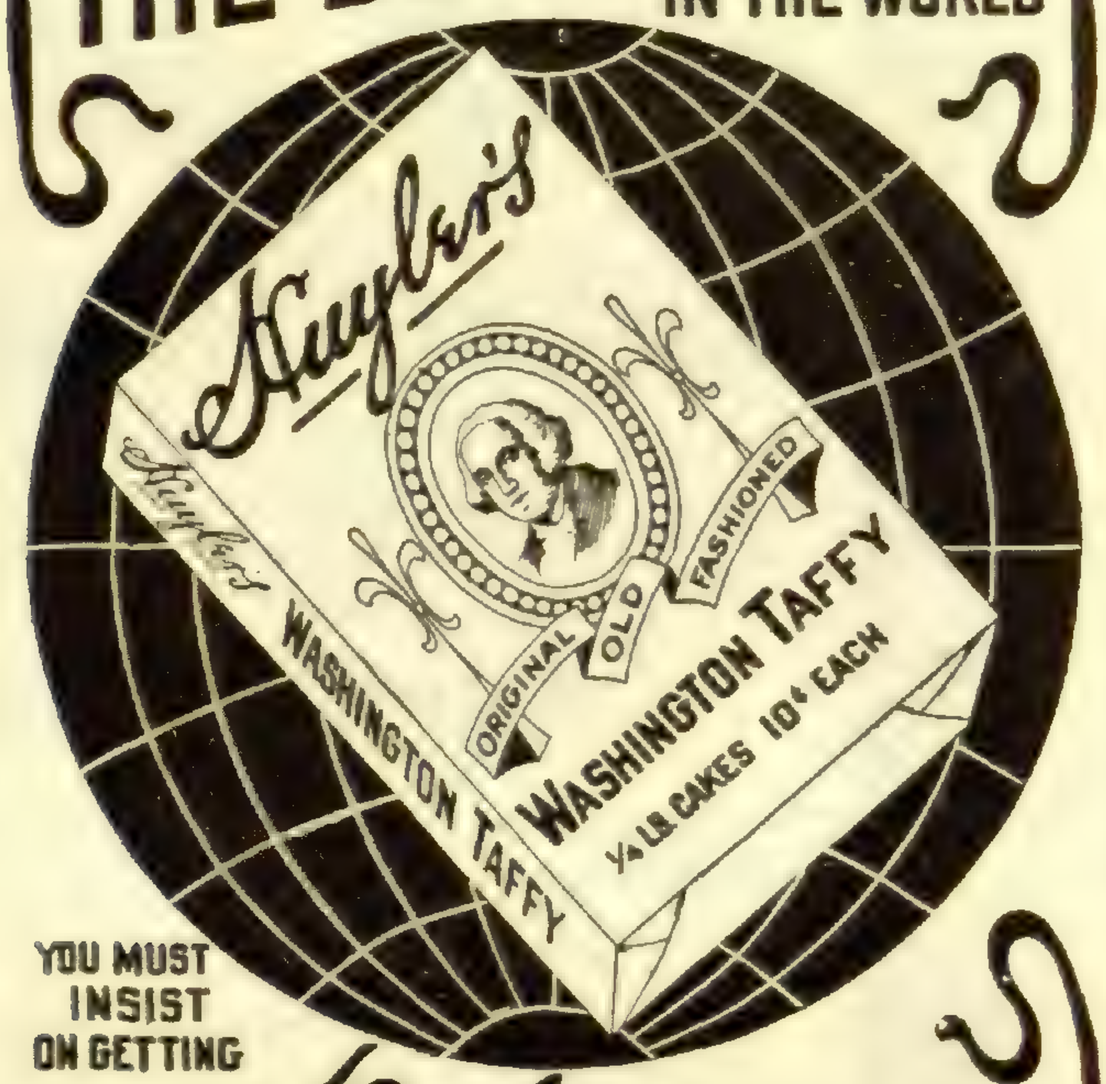

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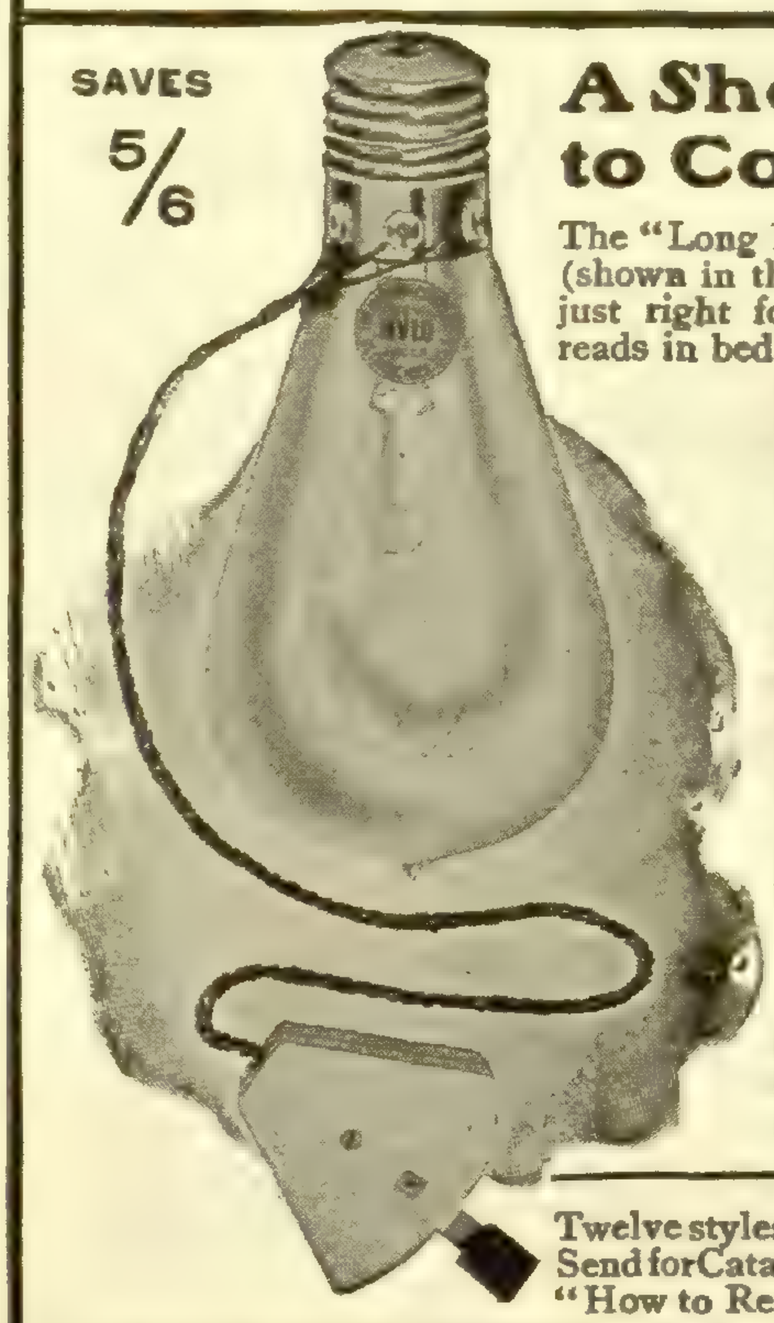

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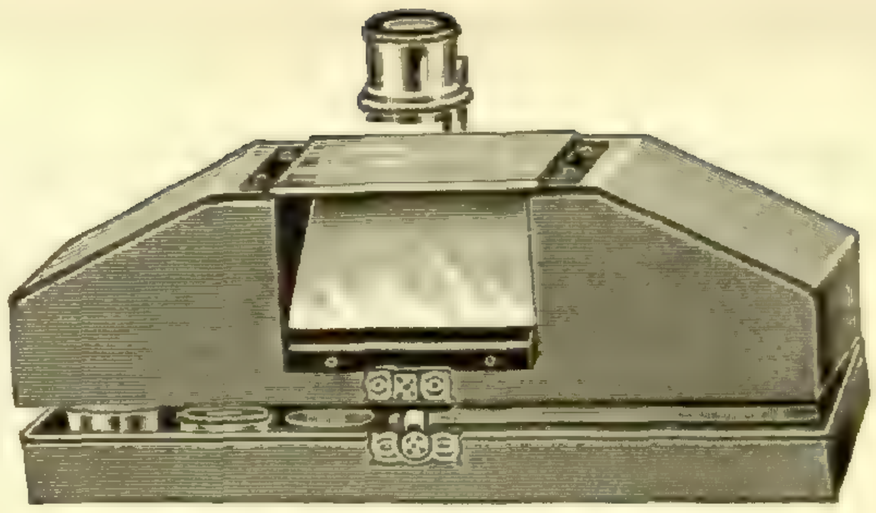

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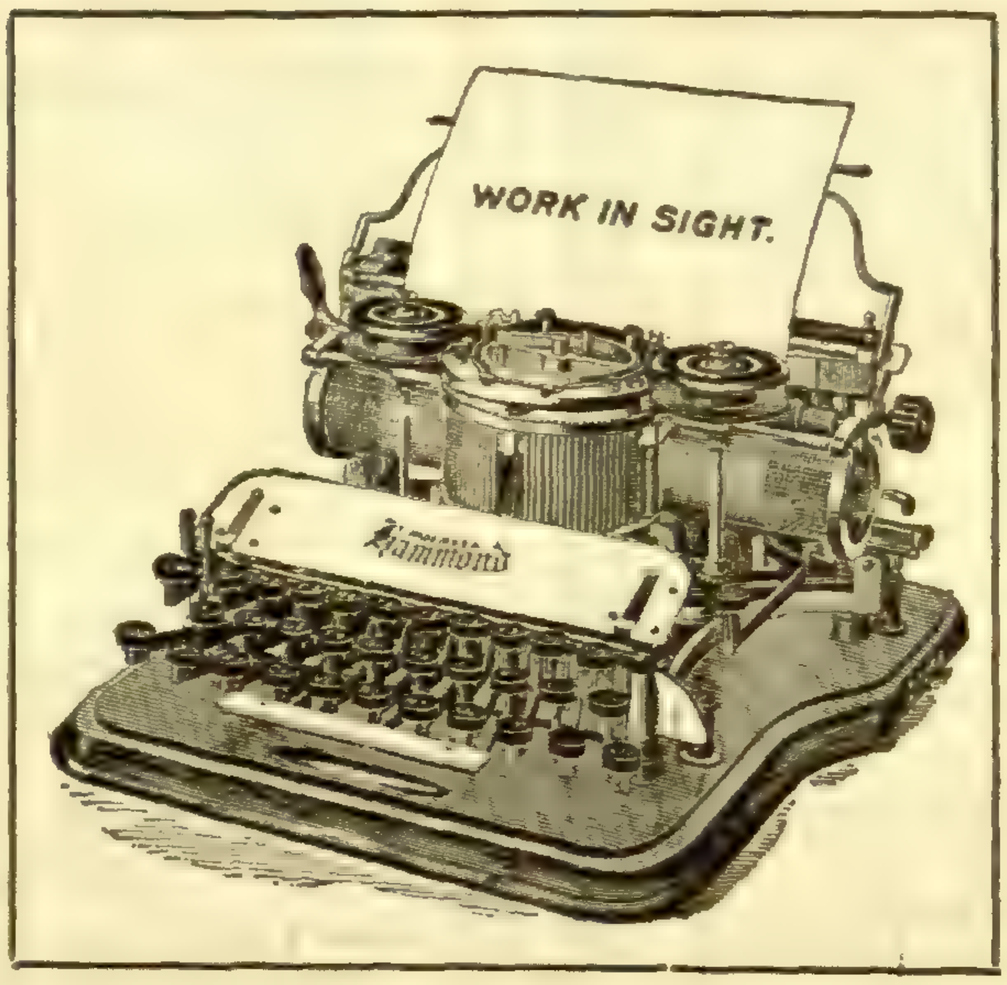

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Editors: JOHN M. COULTER and CHARLES R. BARNES

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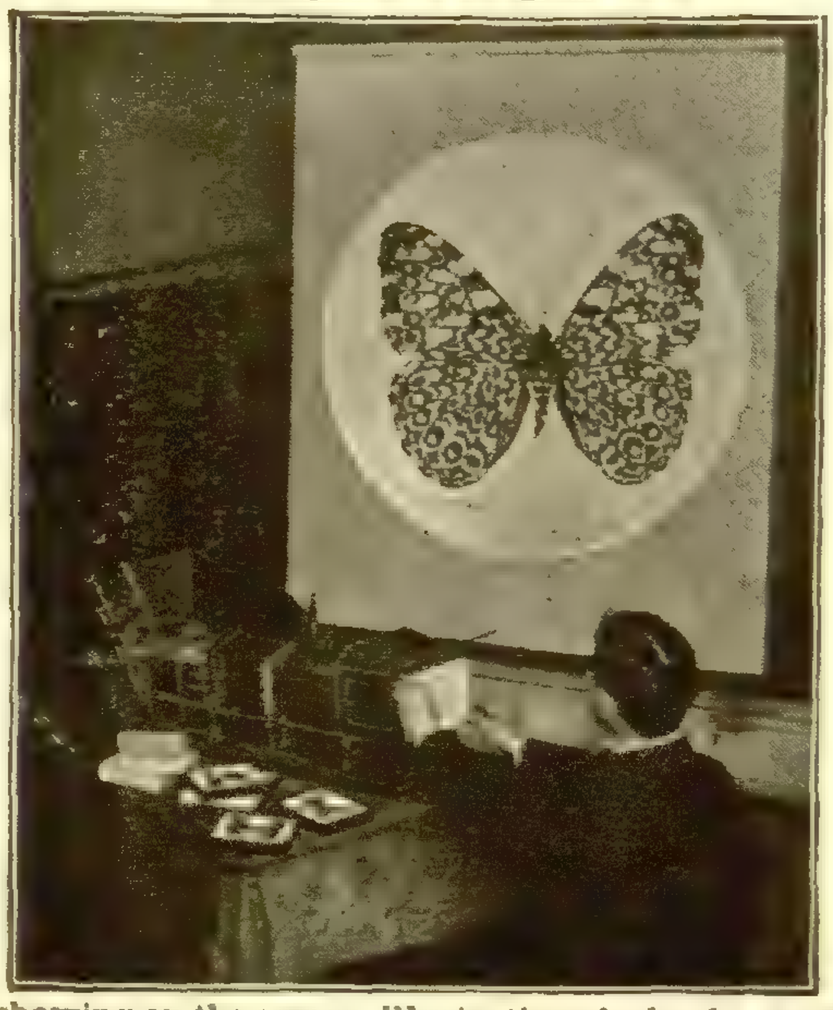

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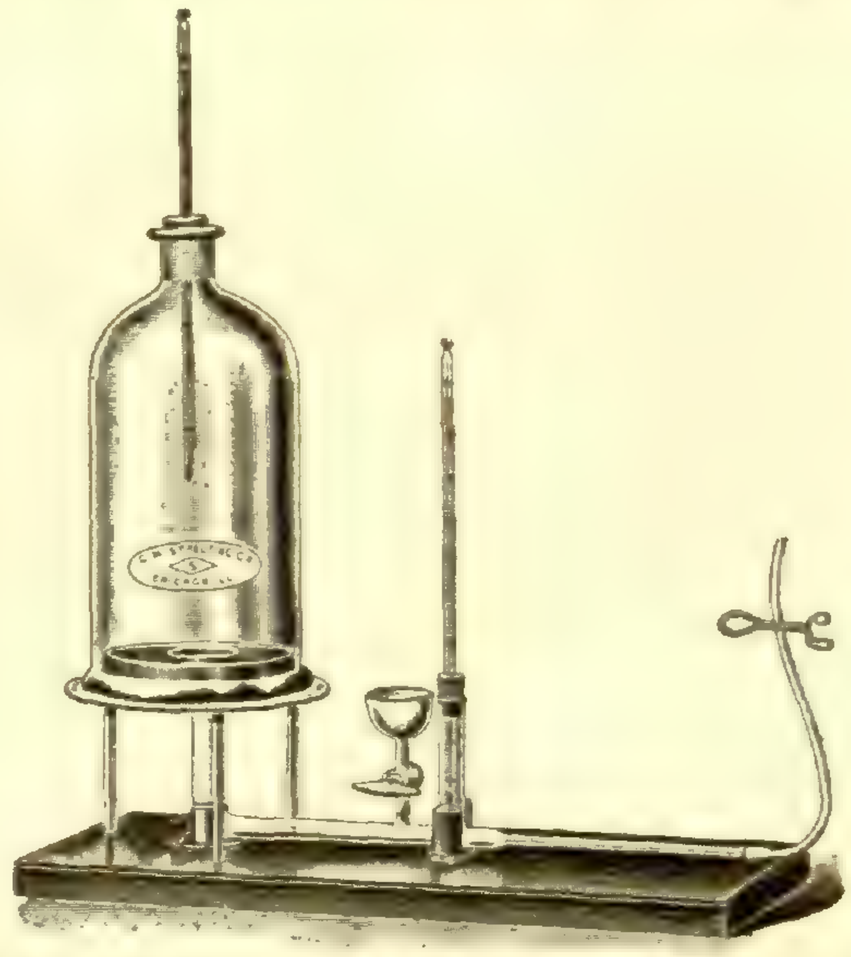

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Trichostema rubisepalum, n. sp.—Erect annual, 2 to 3^{dm} high: stems chiefly branched from near the base, the branches usually in pairs and ascending, soft pilose and glandular, the lower ones becoming reddish: leaves cauline, opposite, entire, subsessile, linear-lanceolate, pilose on both sides and finely glandular, acute or acuminate, 2^{cm} long or longer, about 5^{mm} wide: inflorescence in axillary short-pedunculate cymes; flowers blue, solitary, on 2 or 3 glandular pubescent pedicels, subtended by linear bracts: calyx united below the middle, bristly pilose and somewhat glandular, about 6^{mm} long; the 5 subequal segments straight, acute, ultimately turning to a pink or light purple: corolla exceeding the calyx, 7^{mm} long, curved, pilose, throat oblique; its segments also pilose, thin, obscurely bilabiate; upper lip somewhat shorter and 2-segmented; lower one with 3 segments: anthers much exceeding the corolla, in two pairs of unequal lengths; filaments curved, equaling the tube, slender, glabrous, apparently adnate to the entire length of the thin corolla tube; anther cells united toward the apexonly, attached dorsally to the filament, ovoid, the base ultimately much spreading: style glabrous, filiform, recurved, equaling the shorter stamens and inserted in the depression of the ovary lobes; ovary short pubescent, deeply 4-lobed: seeds amphitropous.

Type specimen collected by Miss *Laura M. Lathrop* at Hernandez, San Benito County, California, August 1902.

This is closely related to *T. laxum* Gray, but distinguished by its long pilose and glandular pubescence, sessile or subsessile leaves, and by its usually pilose corolla. The tips of the sepals soon turn red.

Collinsia Hernandezii, n. sp.—Annual, 10 to 20^{cm} high: stems branched from the base, central ones erect, the outer reclining, soft yellowish pubescent, glandular: leaves cauline, opposite, oblong to oblanceolate, the larger ones 4^{cm} long, 1.5^{cm} wide, apex obtusely rounded, gradually tapering at the base to a 1^{cm} long pubescent petiole, margins entire, short and dirty glandular pubescent on both sides, rather thick, the 3 to 5 obscure nerves parallel; upper leaves

becoming bract-like: flowers large, widely scattered along the spicate racemes, half nodding upon short glandular pubescent peduncles, subtended by leaf-like bracts: the 5 distinct sepals glandular pubescent, 5^{mm} long, 1.5^{mm} wide at base, acuminate: corolla bluish, 2^{cm} long, strongly bilabiate, saccate at base, with gibbose throat; lower lip obscurely 3-lobed, the middle lobe longer and prominently conduplicate; upper lip ascending, shorter, and broadly bilobed: fertile stamens 4, equally inserted upon the tube near the base, longer pair 1.5^{cm} long, shorter pair 2^{mm} less, jointed and papillate at base; filaments winged, downwardly recurved, glabrous or the longer ones glandular above the middle; fifth stamen represented by an oblong membranous pouch on the lower portion of the corolla tube; anthers 2-celled, round or reniform, cells united at apex: ovary soft pubescent and finely glandular; style usually straight, thick, about equaling the stamens, sparsely glandular toward the base.

Type specimen collected by Miss *Laura M. Lathrop* at Hernandez, San Benito County, California, June 1903.

Its habit and pubescence is that of *C. Greenei* Gray, but the leaves and flowers are different.

Fritillaria succulenta, n. sp.—Stems glabrous, erect, simple, 2 to 3^{dm} high, from a bulb of fleshy scales: basal leaves ascending, 5 to 10^{cm} long, 2 to 3^{cm} wide, in pairs or in whorls of three, elliptic-oblong, obtuse, succulent and covered with a bloom; cauline ones few, erect, alternate or the lower in pairs, lanceolate, also fleshy and glaucous: flowers solitary or on the larger plants in racemes of three, nodding; peduncle subtended by a leaf-like bract, glabrous, 1 to 2^{cm} long: perianth campanulate, 2 to 3^{cm} long, wider than that when spreading; the 6 segments 2 to 3^{cm} long, obtuse, oblanceolate to obovate, glabrous beneath, purple, entire, margins at apex yellowish, numerously striate with darker purple and the upper surface pulverulent or obscurely crested: stamens 6, inserted upon the base of the segments, included; anthers 3 to 4^{mm} long, elliptic-oblong, versatile, extrorse; filaments 8^{mm} long, glabrous, more or less expanded toward the base: style 1^{cm} long, glabrous, cleft into three segments half way down, the recurved segments subcompressed and bearing terminal stigmas; ovary smooth, truncate at apex.

Type specimen collected in April 1903 by Miss *Laura M. Lathrop*, at Hernandez, San Benito County, California.

Its leaves are quite thick and fleshy, and are usually glaucous on both sides.

Sanicula serpentina, n. sp.—Low spreading biennial or perennial herb, from slender rootstocks, 2^{dm} high or less, wholly glabrous and frequently somewhat glaucous, a rich brown color when cured: stems chiefly branched from the base, the central one erect, the marginal ones ascending: leaves mostly from the base, subtending the branches, the radical ones upon membranously flattened 3-nerved petioles 2^{cm} long; blade proper 2^{cm} long or longer, ovate in outline, 3^{cm} across the base, pinnately divided into laciniate lobes which are again divided into slender acuminate usually somewhat recurved and sharply pointed segments: inflorescence branched from near the base, long-pedunculate; involucre of sessile leaf-like bracts; heads 3 to 5, the peduncles of the lateral heads usually much shorter at least when in flower, densely flowered, about 4^{mm} in diameter; involucels of entire lanceolate bracts slightly shorter than the yellow flowers; marginal flowers sterile, pedicelled, the fewer inner ones sessile and fertile: calyx 5-toothed: petals 1-nerved, quite broad across the middle, the setaceously acuminate apex strongly inflexed and emarginate on its bend: stamens incurved near the apex; anthers broadly elliptic, 0.5^{mm} long: ovary with uncinate prickles; styles 2, slender, recurved, each persistent from the inner face of the stylopodium: fruit not observed.

Type specimen no. 4498, collected in April 1903 near Portola, San Mateo County, California.

This form is nearest related to *S. laciniata* H. and A., but the latter is a much more rigid herb, with coarser, broader, spinosely toothed leaf divisions; and with the bracts of the involucels often 3-parted or at least 3-nerved from near the middle. It was discovered on serpentine rocks near Searsville Lake, of the chaparral formation.

Trifolium bicephalum, n. sp.—More or less tufted, from an annual root: stems slender, 8 to 18^{cm} long, decumbent or the outer ones prostrate, rather numerous from the base, rarely branched, sparsely pubescent: leaves both radical and cauline, the basal ones somewhat smaller and more numerous, with slender flexuous pubescent petioles 2^{cm} in length; stipules adnate, 6^{mm} long, membranous, strongly nerved, subglabrous or finely ciliate along the edges, terminated by two setae 2^{mm} long; leaflets 7^{mm} long, 4^{mm} wide, soft pubes-

cent with brownish hairs, obovate or truncate and usually emarginate, entire or obscurely dentate above the middle, with prominent ascending nerves beneath: peduncles pubescent, equaling half the length of the stem or branch, subflexuose above the middle; bicephalous heads terminal, sessile, unequal in size, each subtended by a subsessile trifoliate leaf with broad ovate stipules; involucre none: calyx densely pubescent, 4^{mm} long including the 2^{mm} sharply acuminate teeth: corolla exceeding the calyx teeth by 1 or 2^{mm}, hyaline and united with a stamineal tube below the middle; upper lip whitish, obovately rounded and surrounding the lateral lobes or wings; lateral lobes oblongish, slightly shorter than the banner, obtuse or acute apical portion nearly white, the middle portion purple, the basal portion hyaline and with an auricle; keel obtuse, shorter than the wings, purplish: anthers very small: ovary glabrous, 2-ovulate; style glabrous, equaling the stamens, terminally recurved, bearing a capitate stigma.

Type specimen no. 4812, collected at San Pedro, San Mateo County, California, May 1903.

This species comes nearest to the so-called Californian *T. Macraei* H. and A., but is a much smaller and more prostrate clover, with leaves distinctly obovate and emarginate. It forms dense prostrate mats on a high promontory near the sea.

Eriophyllum Greenei, n. sp.—A cespitose perennial, from a woody base: stems many from the crown, lanate, branched above the middle: leaves numerous on the sterile stem, the lower ones opposite, the upper ones alternate, petiolate, triangularly ovate in outline, 2 to 3-pinnately divided, lanose on both sides; the segments short, blunt, very narrow, with incurved margins inclosing a dense matrix of woolly hairs; petiole about equaling the blade, as broad as the segments with edges incurved: heads heterogamous, solitary, terminating the leafy branches, ovoid, 1^{cm} broad; peduncle white tomentose, without bracts; involucre quite rigid, cup-shaped, densely lanate, united at base; bracts in one series, acute, 10^{mm} in length: ray flowers light yellow, 15^{mm} long including the achene, pistillate; tube 2^{mm} long, pubescent, ligule 8^{mm} long, 3^{mm} wide, obovate or oblanceolate, many-nerved, apex obscurely 3-toothed: style arms 1^{mm} long, narrowly flattened, obtuse: receptacle obscurely pitted, somewhat raised and subconic: disk flowers perfect, very numerous,

6^{cm} long with the achenes: tubular corolla yellow, sparsely pubescent, terminated by 5 obtuse segments: anthers 1.5^{mm} long, with apex triangularly ovate, bases obscurely auriculate; filaments barely as long, inserted upon the middle of the tube: style arms flattened, bearing small capitate stigmas: achene brownish black, 3^{mm} long, usually curved and attenuate from the base, subcompressed or 4-angled, edges ciliate: pappus persistent, less than 1^{mm} in length, of unequal paleae.

Type specimen no. 4335, collected in the Mocho Creek Canyon, Alameda County, California, May 1903.

It is intermediate between *E. arachnoideum* Greene and *E. caespitosum* Dougl., but sufficiently distinct from either. Named for Professor E. L. GREENE.

Navarretia Abramsi, n. sp.—Densely lanose herbs, about 6^{cm} high: stems solitary or several from the base, rigidly erect, chiefly branched from the middle; branches rather stout and straight, ascending, terminated by solitary comparatively large heads: leaves mostly subtending the heads, the larger ones 2^{cm} long, 1 or 2-laciniately divided, soon becoming dry and brittle; the lobes very narrow, becoming glabrous, usually recurved and terminated by a fine sharp point: heads turbinate, the larger ones 1^{cm} across at top, densely surrounded by a matrix of lanate hairs, 6 to 10-flowered, the subtending bracts similar to the leaves but smaller: corolla easily separating from the base, 8^{mm} long including the 3^{mm} long segments, bluish or nearly white, glabrous, hyaline, conspicuously nerved; segments 5, subequally divided, linear spatulate, entire or finely dentate at apex: stamens 5, barely exceeding the throat of the corolla, subequal in length, filaments threadlike, subequally inserted upon the tube 2^{mm} below the throat; anthers ovate or elliptic, 1^{mm} long, apex obtuse, base sagittate: style persistent, glabrous, minutely lobed at apex: calyx of the mature capsule 6^{mm} long, divided nearly to the base; the sepals straight and erect, linear, hyaline below the middle, held intact by the hairy matrix; the upper part of the sepals glabrous, foliaceous, and acuminately pointed: capsule triangular, when mature easily falling out from the persistent calyx, straw-colored, smooth and shining, 4^{mm} long, 1.5^{mm} in diameter, apex pointed, 3-celled, loculicidally dehiscent: seeds solitary in each cell, subterete, 3^{mm} long, brown and very hard, with a gelatinous cover which readily dissolves in water.

Type specimen no. 4586, collected on Black Mountain, Santa Clara County, California, July 1905.

It is a very late summer-flowering annual, chiefly confined to dry gravelly soil immediately bordering thickets of the Californian chamiso (*Adenostoma fasciculatum* H. and A.). Named for Mr. L. R. ABRAMS, a former student of botany and classmate at Stanford University.

Ribes Stanfordii, n. sp.—A rigidly branched shrub, 1 to 1.5^{m} high, nearly as broad: bark on the younger branches light brown, becoming grayish white with age, thin, separating into shreds; branchlets subtended and protected by 3 spines, very short and rigid; spines about 1^{cm} long, straight, shining brown, divaricate, distinct, the middle ones usually longer, exceeding the axillary leafy branchlets; branchlets terminated by 1 to 3 small tufts of leaves, subtended by diminutive spines, each tuft provided with a subwhorl of 3 to 5 leaves and terminated by a small inflorescence of 1 to 3 flowers: leaves orbicular, 8^{mm} long including the finely glandular pubescent 2 to 3^{mm} long petiole, deeply cleft into 3 segments, soft pubescent on both sides, rather thick, the segments usually terminated by subequal obtusely rounded teeth or lobes, obscurely 3 to 5-nerved; petiole gradually expanded at base into the adnate stipules: flowers 3, upon a short and pubescent peduncle, each separately inserted and sessile, subtended by conspicuously broad pubescent bracts: calyx about the ovary densely pubescent, 3^{mm} in diameter, its tube 2^{mm} in diameter, less pubescent, about 2^{mm} long, the 5 segments exceeding the corolla by 1^{mm}, triangularly obtuse, puberulous on the outer surface, 2^{mm} long, yellow, rotate or much reflexed: corolla deeper yellow, inserted upon the calyx throat and alternating with the segments, straight, obovate, 2^{mm} long: anthers 5, inserted upon the calyx throat and opposite the segments, equaling the corolla; filaments glabrous, flattened, 1.5^{mm} long: style erect, subterete, slightly exceeding the stamens: anthers ovate, obtuse at apex, light yellow, truncate or only obscurely lobed at base, 1.5^{mm} long, 1^{mm} wide at the base: berry yellow and pubescent at least when young.

Type specimen no. 3958, collected on Mt. Pinos near Griffin's Postoffice, Ventura County, California, July 1902.

It was discovered in open pine regions in the vicinity of cliffs and rocky outcroppings at the summit. Not common. Distributed as *R. nubigenum* McClatch.

Pedicularis Dudleyi, n. sp.—Perennial herbs, 2 to 3^{dm} high, usually from a branched caudex: stems solitary from each of the scaly crowned caudices, the central ones erect, the outer ones ascending, simple or sometimes branched, not exceeding the basal leaves, lanose especially toward the base, more or less curved; basal bracts brown, lanceolate, entire, glabrous, marcescent: leaves chiefly from the base of the stem, alternate, the uppermost at about the middle of the stem but not exceeding it, the lowest ones longest and somewhat decumbent, lanceolate in outline, the larger 25^{cm} long, 6^{cm} wide; leaf segments about 10 pairs, subglabrous or short pubescent on the nerves, membranous; lower pairs distinctly petiolate, the upper pairs not only sessile but broadly united, those along the middle largest; each lobe ovate or oblong in outline, 3^{cm} long, 2^{cm} wide, cleft into irregular lobes or merely dentate, the margins unequally serrate or dentate, its teeth sharply pointed: inflorescence spicate, densely flowered, at most 5^{cm} long and 3^{cm} in diameter, upon peduncles equaling half the length of the stem, usually erect but frequently somewhat curved; bracts subtending the flowers, foliaceous, serrately toothed, the upper ones equaling the flowers, the lower ones much exceeding them: calyx 1^{cm} long, unequally 5-cleft, the segments acute and obscurely toothed toward the apex, densely lanose on the exterior: corolla 2^{cm} long, the narrow tubular part half that in length, glabrous; upper lip conduplicate, slightly notched at apex, pink or whitish, much protruding and arched; lower lip subequally 3-toothed: stamens equal; filaments glabrous; anthers broadly elliptic, attached to the basal dorsal side, the cells connate and rounded at apex, the base not united, acute: style filiform, subpersistent, thicker and more or less flattened toward the apex, conspicuously recurved and protruding from the upper lip; ovary glabrous, dark brown, 2-celled, flattened, acuminately pointed: capsule coriaceous, 12^{mm} long, 7^{mm} wide, acuminately terminating in an upwardly curved point: seeds about 4, black when mature, pitted, subterete or obscurely angular.

Good flower and fruit of this type specimen, no. 4289, was collected in May and June 1903, at Iverson's Ranch on the Pescadero Creek, San Mateo County, California.

Only known from this locality, where it is rare and confined to the deep shade of *Sequoia sempervirens*. Its proximity to a camping ground endangers its exist-

ence. This denizen of the Santa Cruz Mountain redwoods is named in honor of Professor W. R. DUDLEY of Stanford University, who first discovered it.

Orthocarpus longispicatus, n. sp.—A profusely branched decumbent biennial or perennial, forming rather dense mats: stems slender, elongated and distantly branched, often 1^{m} in length, usually pubescent with soft glistening white hairs: leaves alternate, evenly scattered, sessile, membranous, puberulent on both sides, or with glistening hairs on the margins and along the 3 obscure nerves, cleft into 2 pairs of strap-like segments, the middle one longest: inflorescence spicate, very long and usually curved; bracts not exceeding the flowers, 5 to 7-laciniately cleft, the obtuse apices light red: calyx 4-cleft, soft pubescent, equaling the corolla, with colored tips: tube of the corolla 2^{cm} long, externally pubescent, gradually expanded from the constriction above the ovary; upper lip 1^{mm} longer than the lower one, rather straight, apex obtuse, finely pubescent on the lower surface, margins soft and hyaline; lower lip with 3 obtuse finely pubescent teeth which bear moderate sized sacs: stamens 4, inserted upon the middle of the corolla tube, the lateral pair shorter, the upper pair nearly equaling the galea and enclosed by it; filaments hyaline, linear-flattened, glabrous; the upper anther cell usually somewhat longer and shedding its pollen before the lower one: style persistent, glabrous, much exserted, thickened or expanded toward the base of the capitate flattened or obscurely lobed stigma; ovary oblong, truncate at the apex: capsule 10^{mm} long, smooth, loculicidal: seeds numerous, lenticular, with broad reticulate wings.

Type specimen no. 4938, collected in July 1903, at Point Reyes, Marin County, California.

It was quite abundant among the pickle weed (*Salicornia ambigua* Michx.), along edges of brackish water. Distinguished by its long decumbent fragile stems and branches, numerous leaves, and elongated densely flowered spikes.

Godetia lanata, n. sp.—Erect annual, 3 to 6^{dm} high, single or branched from near the base, quite rigid; mature stems shining, straw-colored, scaling at base into membranous shreds, with ascending branches from or above the middle; the younger branches yellowish tomentose: leaves cauline, lower ones soon falling, alternate and clustered, sessile, very unequal, cinereous on both sides, semicoriaceous, lanceolate or linear-oblong, equally tapering at both ends, acute, the larger ones 5^{cm} long, 15^{mm} wide, midnerve quite promi-

nent beneath, lateral ones obscure: inflorescence short, spicate or subcapitate, terminating the branches, usually densely flowered, 3 or 4^{cm} in diameter; buds erect, from the terminal central axis; flowers easily separating from the ovary, subtended by strigose lanceolate acuminate bracts, subsessile: calyx tube obconic, 3 to 4^{mm} long, lanose pubescent; its 5 equally pubescent segments 12^{mm} long, acuminate and ultimately reflexed: corolla and stamens inserted upon the rim of the calyx throat; petals straight, thin, pink, broadly obovate in outline, 6^{mm} long and wide, irregularly or obscurely 3-toothed, the middle tooth acute, usually the larger: stamens 8, in 2 series, those alternating with the petals nearly equaling them, those opposite the petals barely more than 1^{mm} long; filaments glabrous, compressed, broadest at the base; anthers introrse, basifixed, those of the upper series twice as long as those of the lower: style glabrous or with a few long hairs, barely equaling the stamens, bearing an obscurely 4-lobed stigma; ovary densely and persistently lanose pubescent, upon short thick pedicels: capsules subtended by leaf-like bracts longer than themselves, loculicidally dehiscent from the apex, straight, erect, lanose, subsessile, apex truncate, nearly of the same thickness throughout, subterete or only slightly 4-sided, 8-costate, 4-celled, 4-valved: seeds numerous, in single rows, dark brown, subterete or cubical, a little pointed at one end.

Type specimen no. 4376, collected in June 1903 at Bardins railroad switch, Monterey County, California.

This characteristic species was found quite plentiful on the sandy plain between Monterey and Castroville, and is quite variable in the density of its pubescence branching habit, and size of leaves.

Pentachaeta laxa, n. sp.—A lax very much branched annual, 1 to 2 or 3^{dm} high: stems branched from the base, softly but sparsely pilose: leaves in pairs, subtending the branches, sessile, linear, gradually tapering from the base, the larger ones 3^{cm} long, 2^{mm} wide, very thin, sparsely pilose on both surfaces: heads terminal, heterogamous, turbinate, 6^{mm} long, about 9-flowered; the peduncle ascending, 1 to 4^{cm} long, pilose, somewhat thickened toward the apex; involucral bracts 3 to 5, persistent, flat, acute, oblong, scantily pilose on the exterior, the reticulate nerves quite prominent, equaling the flowers, more or less membranous: receptacle pitted: each of the ray flowers

subtended by an involucral bract, yellow, caducous, pistillate, tube 1^{mm} long, bearing a broad 1^{mm} long notched ligule: style arms exceeding the ligule, recurved, acute: disk flowers perfect, tubular, light yellow and caducous, 2^{mm} long, the upper half inflated, bearing 4 obtuse teeth: anthers well included, 0.5^{mm} long, rather broad, bases truncate, each with a very prominent apical appendage; filaments thread-like, scarcely longer than the anther: style barely exceeding the corolla, its arms subcompressed, recurved, short, obtuse or truncate at apex: achenes subterete, 5^{mm} long, black when mature, finely rugose, dotted with sessile yellowish brown glands subtended by short setae; pappus of 2 or 3 paleaceous scabrous awns, usually persistent.

Type specimen no. 4437, collected in May 1903, on Cedar Mountain of the Mount Hamilton Range, Alameda County, California.

This distinct species inhabits a steep shaded ravine of fertile soil, and forms a tangled mass with its numerous decumbent branches. Not observed elsewhere, and evidently very rare.

Nemophila Fremontii, n. sp.—Delicate annual: stems procumbent or prostrate, branched, subglabrous or sparsely retrorsely pubescent, 10 to 30^{cm} in length: radical leaves similar to the lower cauline ones, frequently forming a rosette, 3^{cm} long including the 1.5^{cm} long strigose petiole; blade membranous, ovate or oblong in outline, usually oddly pinnate with two pairs of nearly divided lobes or the uppermost merely sinuate, sparsely pubescent on both sides, paler beneath; the lobes nearly as broad as long, rounded, finely mucronate: flowers solitary, upon slender flexuose usually recurved 2^{cm} long peduncles which are clothed with retrorse bristles: calyx persistent, campanulate, 3^{mm} long, the basal one-third united, equaling or exceeding the corolla, pubescent with fine bristle-like hairs; sepals oblong, obtuse or acutish, foliaceous, with a short blunt recurved appendage from each sinus: corolla white, urn-shaped, its lobes becoming reflexed, at most 3^{mm} long, 5-cleft, the basal appendages quite obsolete; petals hyaline, ovate to oblong or obovate, obtuse, sparsely ciliate on the edges above the middle: anthers 5, alternate with the petals, erect, quite a little shorter than the corolla; filaments inserted half way down on the corolla tube, glabrous, 1^{mm} in length; anthers 0.3^{mm} long, comparatively broad, apex obtuse,

base subcordate: ovary sessile, densely pubescent: style 1^{mm} long, cleft into 2 recurved arms, terete, glabrous, persistent; stigma terminal, capitate: capsule 4^{mm} in diameter, globular, sparsely ciliate: seeds compressed, carunculate.

Type specimen no. 4901, collected in May 1903, on Fremont's Peak of the Gabilan mountains, San Benito County, California.

It was observed only at the very summit of the peak, among the moss-covered rocks.

Monardella franciscana, n. sp.—A sprawling suffrutescent perennial: lower stems reclining on the ground or in dense herbaceous thickets, woody, one or more meters long; the leaf-bearing upper stems usually clustered, herbaceous, woolly pubescent, 2 to 3^{dm} long, erect or decumbent near the base: leaves opposite, mostly fascicled, very variable in size, densely woolly pubescent on both sides especially beneath; the larger upper ones 2 to 3^{cm} long including the 5^{mm} long petiole, 2^{cm} wide near the base, broadly ovate, entire or with a few obscure teeth, the edges recurved; the lower or axillary ones sessile, lanceolate to elliptic-obovate: inflorescence capitate; heads densely flowered, terminal, rarely more than one, 3 to 4^{cm} in diameter, subtended by a whorl of pubescent leaf-like bracts equaling or exceeding the flowers; flowers upon stout short pedicels: calyx about 8^{mm} long, the marginal ones usually curved upward, conspicuously 11 to 15-nerved, silky pubescent except near the base, tubular, equally 5-toothed; the teeth acute, 1.5^{mm} in length: corolla blue or light pink, funnel-shaped, the longest 2^{cm}, strigose about the middle, glabrous toward the base, bilabiate; upper lip 5^{mm} long, erect or straight, apex 2-lobed; lower lip divided into 3 linear segments, equaling the upper lip, usually deflexed: stamens 4, fertile, moderately unequal, exserted and spreading; filaments slender, glabrous, inserted at the corolla throat or a trifle beneath it; anthers attached to the base, the cells somewhat recurved: style equaling the stamens, glabrous; stigma minute, terminal; ovary glabrous, distinctly 4-lobed.

Type specimen no. 4766, collected at San Pedro, San Mateo County, California, July 1903.

It was repeatedly observed in dense herbaceous growths in the ravines on the coast from San Francisco to Santa Cruz, and is a distinct seacoast species.

HORKELIA BOLANDERI **marinensis**, n. var.—Tufted perennial, 1 to 2^{dm} high, villous pubescent: stems deflexed and more or less decumbent: leaves pinnately divided into 5 to 8 pairs of leaflets, subradical though often the stem bears as many as 3 to 5 leaves without subtending branches; average leaflets 10^{mm} long, unequally 4 to 10-toothed; basal stipules narrowly linear, 10^{mm} long, those on the stem broader and usually 1 or more-toothed: inflorescence branched from near the middle, corymbosely paniculate; branchlets loosely flowered: hypanthium longer than wide, saucer-shaped; bracts broad, 3-toothed: stamens 10, opposite the outer and inner series of sepals which must exceed them: outer sepals shorter, spatulate or acute at apex, pubescent on both sides; inner ones acuminately triangular, glabrous on the inner surface: filaments glabrous, flattened, slightly unequal in length; anthers introrse, short, elliptic: petals white, narrowly spatulate or oblanceolate, about equaling the sepals, early falling: styles many, glabrous, erect: seeds bean-shaped, smooth.

Type specimen no. 5039, collected in sandy soil at Point Reyes, Marin County, California, July 1903.

This variety has been distributed as *H. Parryi* (Wats.) Ryd. It has the pubescence of typical *H. Bolanderi* Gray, but the stems are more or less decumbent, basal stipules strictly linear and almost twice as long as in the species, leaflets larger and more toothed, and inflorescence more loosely corymbose and with larger hypanthia.

Chrysopsis arenaria, n. sp.—A wiry perennial herb, from creeping and much branched roots: stems 10^{cm} long or very much shortened and bearing a dense rosette of leaves, erect or more often subreclining, hirsute: basal leaves soon withering; cauline ones many, ascending, obovate to oblanceolate, the lower ones slenderly attenuate from the base, alternate, entire, hispidly hirsute on both sides, ascending: heads heterogamous, terminating the stems, 1 to 3, barely exceeding the leaves, upon densely hirsute often glandular and few-bracteolate peduncles, 15^{mm} in diameter, nearly that in length; involucre of several series, campanulate; bracts linear, imbricate, acuminate, pubescent, the longer ones 10^{mm} in length and usually with pink scarious margins: receptacle flat, favose: ray flowers in one series, pistillate, showy; ligule yellow, 4-nerved, narrowly oblanceolate, entire, 6^{mm} long; its tube slender, 5^{mm} long, glab-

rous, expanded at base: style barely exceeding the throat, with short appendages: disk flowers many, all tubular, equaling the pappus, perfect, terminated by 5 acute teeth, 6^{mm} in length, expanded at the base, yellow: anthers included, 2^{mm} long, bases obscurely auricled, apex triangular, acute; filaments glabrous, inserted upon the tube one-third from the base: style much exceeding the tube, its arms flattened, short and truncate: achenes compressed, silky pubescent: pappus bristle-like, chiefly of two series, rusty or yellowish white, the longer series 5^{mm} in length, finely scabrous, the basal one very short, lighter colored, smooth.

Type specimen no. 4556, collected at Point Reyes, Marin County, California, July 1903.

It forms dense prostrate mats on the windward side of the drifting sand dunes.

Castilleia Wightii, n. sp.—A tufted perennial herb, 3^{dm} to 1^{m} tall: stems several from the crown of the root, much branched from the base to the middle, glandular pubescent with dirty yellowish hairs: leaves alternate, sessile, quite membranous, pulverulent on both surfaces, or sparsely hairy along the 3 nerves, broadly linear to oblong, the larger ones 6^{cm} long and 15^{mm} wide, mostly with one pair of linear lateral segments from the middle of the leaf, the terminal lobe much longer and wider: inflorescence spicate, 10 to 20^{cm} long, terminating the erect corymbosely disposed branches; subtending bracts barely equaling the flower, densely covered with glistening subglandular hairs, 3-nerved and 3-cleft, the upper ones with colored tips: calyx pubescent, laterally compressed and equally cleft nearly to the middle, the halves 2-toothed, subequaling the corolla: tube of corolla proper 10^{mm} long, glabrous, saccate, its nerves prominent; galea or upper lip longer than the tube, quite broad and membranous at base, straight, its blunt apex rather thickened and retrorsely pubescent on the outer surface; lower lip 3-toothed, teeth 1^{mm} long, obtuse, all alike: stamens equally inserted upon the middle of the corolla tube, the lateral pair a trifle shorter, the upper pair nearly equaling the galea and enclosed by it; , filaments glabrous, flattened, with hyaline margins; anther cells broadest at base, subequally attached: style little protruding, flattened, glabrous, persistent, often recurved near the apex, bearing a capitate or obscurely flattened stigma; ovary smooth, somewhat compressed, acute toward the apex: cap-

sule 12^{mm} long, ovoid, sharply pointed, coriaceous, loculicidal: seeds many, orbicularly compressed or somewhat elongated, with reticulate wings.

Type specimen no. 4761, collected on the trail between Spring Valley Lake and San Pedro, San Mateo County, California, July 1903.

It is a uniquely distinct subglandular species easily recognized by its abundant foliage, densely flowered elongated spikes, and broadly linear unequally 3-segmented leaves. Named for W. F. WIGHT, a former student of botany and classmate at Stanford University.

Phacelia flaccida, n. sp.—Delicate annual, 3 to 6^{dm} high: stems sparingly but widely branched from the middle, rather weak and more or less reclining, rarely erect, beset with stinging white hairs: leaves few, alternate, usually subtending the branches, the larger ones 5^{cm} wide and 7^{cm} long, ovate to elliptic in outline, very thin and flaccid, only sparsely setigerous on both surfaces, 3 to 5-pinnately nerved, obscurely lobulate, the margins with irregular low roundly obtuse teeth, base rounded or obscurely and unequally subcordate; longer petioles 4^{cm} in length, setose, the younger ones cinereously pubescent: inflorescence usually dichotomously branched; flowers secund, not crowded, upon short ciliate pedicels: calyx of 5 persistent herbaceous distinct segments, the 4 smaller ones oblanceolate and in the mature state 7^{mm} long, bristly ciliate: corolla barely exceeding the calyx, 3^{mm} long, blue or pale white; the 5 segments short-ovate and comparatively very broad: stamens well included within the tube, alternating with the segments and inserted near the base of the tube; anthers short-elliptic, versatile; filaments nearly equal, glabrous, usually curved above the middle, subtended by small hyaline entire membranous folds: ovary persistent, pubescent; style persistent, equaling the anthers, cleft almost to the base, each division bearing a minute terminal stigma: capsule ovoid, 1-celled, divided into 2 valves, 1-seeded in each; seeds brown, flattened, 2^{mm} long, obscurely pitted.

Type specimen no. 4404, collected at Wright's Station, Santa Clara County, California, June 1903.

This delicate species was discovered in a damp deeply shaded nook on the banks of Los Gatos Creek. Otherwise not known.

Leptosyne Hamiltonii, n. sp.—Glabrous acaulescent annual: leaves tufted, all radical, mostly erect, rather thick, 2 to 3^{cm} long

including the 1 to 2^{cm} long flattened petiole; blade proper triangular-ovate in outline, 1^{cm} long, fully as wide at base, 2 to 3-pinnately divided; the segments not wider than the petiole and the rachis, 1^{mm} wide, ultimate ones short, with bluntly rounded apices: scapes usually 1 to 3 from each root, glabrous, 10 to 18^{cm} long, slender, usually somewhat curved, each bearing an erect solitary head; heterogamous heads subcampanulate or cup-shaped, 1^{cm} in diameter, a trifle less than 1^{cm} in height; involucre glabrous, truncate and united at the base, in two series; bracts of lower series 5, thickish, linear, obtuse, 4^{mm} long, dark brown; inner series of 8 to 10 striate bracts, shining, yellowish, about 10^{mm} long, 4^{mm} wide, submembranous, oblong, acute: ray flowers about a dozen or more, pistillate and fertile; tube 1.5^{mm} long, subglabrous; branches of stigma barely exserted, recurved; ligule 3 to 4^{mm} wide, 6^{mm} long, elliptic to oblong or obovate, apex round, usually with a short obtuse tooth: scales equaling the flowers, subpersistent, linear, hyaline, obtuse, 3-nerved, those of the central flowers narrower: central flowers crowded, perfect, sterile, tubular; tube 4^{mm} long, hyaline, cylindric, the upper half subinflated, its 5 teeth obtuse and more or less thickened along the margins: anthers linear, 2^{mm} long, truncate at base, apex triangularly appendaged: style equaling the stamens, its 2 branches short, obtuse, and flattened; achenes linear-obovoid, compressed, marginally winged, ciliate on the edges, pubescent on the sides, brown and glabrous when mature: pappus of 2 caducous hyaline finely ciliate membranes.

Type specimen no. 2328, collected on Mt. Hamilton, Santa Clara County, California, April 1900.

It was in fine flower and fruit, and grew in dry gravelly soil on a steep slope a few hundred yards below the observatory. Since then I have failed to find it either in this same place or elsewhere.

EUNANUS ANDROSACEUS Curran.

This southern species was originally discovered at Tehachapi, California. From the middle western part of the state it is only known at Ben Lomond, Santa Cruz County, where fruiting specimens were collected by Mrs. *K. Brandegee* in April 1890. In July 1903 the writer found excellent flowering specimens in the same locality, which were distributed under no. 4519. It is evidently rare and prefers hot and dry gravelly soil of the chaparral formation. The plants were from 1 to 10^{cm} high, with single or branched stems, glandularly pubescent or the

older ones subglabrous: leaves sessile, obovate or linear-oblong, entire or obscurely apiculate above the middle, apex obtuse, attenuate toward the base, 5 to 20^{mm} long, the larger ones 5^{mm} wide: pedicels of solitary axillary flowers not exceeding 5^{mm} in length: calyx 8^{mm} long, compressed, somewhat inflated below the middle; the two upper parallel lobes a trifle longer than the lower and lateral ones, with 5 short and obtuse teeth: crimson corolla little exceeding the calyx, slenderly tubular, conspicuously constricted below the limb, pubescent on the exterior; limb barely bilabiate, the broadly rounded segments rotately spreading: stamens 4, all anther-bearing; filaments slender, inserted upon the corolla at the middle; anthers 0.5^{mm} long, comparatively broad, widely spreading: style glabrous, erect, bearing flattened lobed stigmas; ovary smooth, conically elongated.

Lupinus polyphyllus Lindl.

This magnificent seacoast lupine was found as far south as San Pedro, San Mateo County, California, May 1903. At this station it grew in wet adobe soil, reached a height of 2^{m}, and is succulent and apparently an annual. The larger leaves were of an enormous size, and some of the flowering spikes exceeded the length of a man's arm. Flowers large, dense, with blue wings and purplish banner. Pods persistent, densely covered with soft long yellowish-white hairs. Distributed under no. 4444.

Campanula exigua Rattan.

No. 4357 was collected in May 1903 along La Puerta Creek, Stanislaus County, California. It was found on a dry mountain side composed chiefly of small rocks, and on gravelly embankments of the creek. At first it reminded me of a Gilia in growth and habitat. The flowers are large and showy: corolla bluish white, at first appearing tubular, ultimately campanulate: styles wholly included, bearing 3 revolute stigmas; dilated bases of the filaments not ciliate; capsule dehiscing regularly on the sides, 3-celled; seeds numerous, 0.75^{mm} long, half as wide, compressed, shining light brown, inserted upon 3 conspicuously enlarged placentae centrally attached to the main axis.

Salix Breweri Bebb.

The type of this willow was collected by W. H. Brewer on San Carlos Mountains of middle California, and described in Bot. Calif. **2**:88. 1880. Since then it has been reported from only a few localities. In May 1903, while making a botanical trip from Livermore, California, through San Antonio and Adobe valleys, I found it in fine fruit, and distributed it under no. 4648. It is quite abundant along the Little Colorado, Sweet Water Creek, and on the headwaters of the La Puerta, all of which rise on Red Mountain, Santa Clara County, California. This species was at once distinguished from the other willows, and bears a remarkable similarity to *Salvia mellifera* Greene.

It is a shrub 1 to 2^{m} high, rather gracefully branched from near the base;

branchlets sparsely branched, erect, about as tough as the western leatherwood (*Dirca occidentalis* Gray), cinereous pubescent: leaves terminally clustered, subsessile, linear to oblanceolate, acute, the larger ones 6^{cm} in length and 10^{mm} in width, densely canescent beneath, upper surface green and becoming glabrous except the sunken cinereous midnerve, margins entire or sometimes remotely apiculate: fruiting spikes immediately beneath the foliage, the short peduncle subtended by 3 foliaceous bracts, curved and slenderly elongated, 5 to 7^{cm} long, 10^{mm} in diameter, densely flowered: capsules short-canescent all over, 5^{mm} long including the very short pedicel, conspicuously compressed, lateral face ovoid, acuminate; styles persistent, not longer than 1^{mm}, 2-cleft, each segment again divided into recurved lobes: bracts spatulate or obovate, long ciliate pubescent; axillary gland persistent, quite prominent and cinereous.

CUPRESSUS GOVENIANA Gord.

About sixteen miles southeast of Livermore, Alameda County, California, is a mountain commonly known as Cedar Mountain. While collecting on this mountain during the latter part of May 1903, I did not find any true cedars, but found near the round-topped summit quite an area densely covered with a species of cypress. It was too late for staminate flowers, but an abundance of mature cones was collected. The trees are mostly of a young generation, of all sizes from 3^{dm} to 5^{m} high and cone-bearing, widely branched from near the ground, the terminal portion of the stem much exceeding the shortest uppermost branches; leaf branchlets slender, more or less spreading: leaves without conspicuous dorsal pits, acute, shining green: cones globose, 13^{mm} in diameter; the 8 unequally sized scales grayish-white on the outside, almost smooth or only with low umbos: seeds angular, prominently margined or winged along the lateral edges above the middle, reddish-brown and frequently somewhat glaucous, with a resiniferous odor.

This species was distributed under no. 4487. There is some doubt as to this determination, and its relationship to the other closely allied species is not as yet clearly understood. These flourishing trees might have been planted at some very early date.

MUHLENBERGIA DEBILIS Trin.

The distribution of this handsomely tufted grass is given from Texas to southern California. In May 1902 the writer found it in abundance on a dry gravelly hillside near Santa Barbara, California. In June 1903 a few dwarfed specimens were discovered on a cliff at Carmel Bay. The latter locality is its most northern known limit along the coast.

GOVERNMENT LABORATORIES,
Manila, P. I.

SOME LITTORAL SPERMATOPHYTES OF THE NAPLES REGION.

J. Y. Bergen.

The strand flora about the Bay of Naples differs so much from most of those which have been studied with reference to the toxic effect of sodium chlorid solutions that it seemed to the writer worth while to investigate the conditions of existence of a characteristic association.

Along a strip of beach sand not quite two meters above the average sea level and less than ten meters from the water line, on the margin of the Bay of Baiae, a well defined association of somewhat more than fourteen members was found. All of these occurred within a distance of a hundred meters, measured along the shore. The species determined were:

Euphorbia Paralias, E. terracina, Polygonum maritimum, Matthiola sinuata, *Alyssum maritimum, Plantago Coronopus, Medicago marina, M. litoralis, *Lotus ornithopodioides, Eryngium maritimum, Echinophora spinosa, *Senecio vulgaris, *Artemisia variabilis, *Inula viscosa.

I shall refer to this group as Association A. Other species occur as members of the association, but none could be identified with certainty at the time when these studies were made (January, February, and March). The five species designated by asterisks are much more abundant in inland stations than they are as strand plants. Conspicuous members of other strand associations neighboring the one above given are: *Narcissus Tazzetta* var., *Thymelaea hirsuta*, *Glaucium flavum*, and *Verbascum sinuatum*. Out of the fourteen listed above, *Euphorbia Paralias*, *Polygonum maritimum*, *Eryngium maritimum*, and *Echinophora spinosa* are the most notably psammophilous species. All four of these are capable of growing out of drifting sand, emerging again and again as they are partially buried.

Only a few of the structural peculiarities of halophytes, as enumerated by Warming,[1] were to be noted in the association under discussion. Studies carried through a considerable part of the year

[1] Oekologische Pflanzengeographie. Zweite Auflage. Berlin. 1902. pp. 305–308.

would be necessary in order to enable one to make a detailed comparison between plants of the same species found growing as members of this strand flora and further inland. The points that could be established during the months of late winter and early spring when these observations were made were as follows:

Alyssum maritimum (littoral form): leaves with more, longer, and stouter hairs on both surfaces than occur on the ordinary form; leaves much smaller and darker green than in the ordinary form; leaves from 1.5 to 3.5 times as thick as in the ordinary form.

Senecio vulgaris (littoral form): plants very low, often with the leaves in rosette form and heads in full bloom only 2^{cm} high. The maximum height observed was 8^{cm}, while the average height of plants growing in fairly good soil further inland was (ten specimens taken at random) 35.7^{cm}. The stems of the littoral form were proportionately stouter and more hairy, and the leaves were darker green, more pubescent, thicker, more sessile (sometimes clasping and almost decurrent). The root system of plants of the littoral form is much more developed than in the ordinary form, but there are no aerial roots, such as are commonly found along the lower part of the stems of this Senecio when growing in ordinary situations.

Artemisia variabilis (littoral form): leaves with more slender divisions, the tips more strongly mucronate and the surface more scaly than the ordinary form; the young leaves of littoral plants were often densely pubescent, while I have never seen them so on plants growing in ordinary stations.

No decided differences were noted between the specimens of Inula found growing in the beach sands and those occurring elsewhere. The Lotus plants were too young to be compared with inland specimens.

The beach sand in which Association A was growing consisted of grains for the most part ranging from 0.80^{mm} to 0.30^{mm} in diameter. It was evidently mainly derived from comminuted trachyte and scoriaceous lava, probably from the little extinct volcano of Monte Nuovo close by. A sample of the sand was taken February 1 from among the roots of the *Euphorbia Paralias* and *Matthiola sinuata* and analyzed gravimetrically for chlorin. It contained 7.3 per cent. of moisture, which contained a trifle more than 0.04^{mg} chlorin

per gram. In round numbers, 0.2 per cent. of the moisture was sea water. This extremely low per centage of chlorin accords with the fact that waves never in ordinary storms wash up as far as the level on which the plants were growing. They must, however, sometimes break into spray which reaches the station of the association studied. For the purpose of getting data for comparison of the salinity of the sand above described with other marine sands and soils and with ordinary garden soil, I made some further analyses, which are summarized in the table below.

	Per cent. Cl	Per cent. sea water	Per cent. water	Sea water per cent. of total water
1. Beach sand, Bay of Baiae (Association A)	0.0003	0.015	7.3	0.19
2. Beach sand, Mare Morto	0.0163	0.807	21.4	3.76
3. Beach mud, Lake Lucrinus	0.0125	0.619	52.5	2.23
4. Loam from vineyard near Pozzuoli	0.00025		25.0	

The second column of the table shows what proportion of sea water present in the sand or mud would account for the amount of chlorin actually found. The third column gives the total moisture present, and the fourth column shows what proportion of total water in the soil was sea water. The calculations are based on the assumption that the water of this portion of the Mediterranean contains in 1,000 parts about as follows:[2] NaCl, 28.76; KCl, 0.66; MgCl, 3.25.

In the beach sand from Mare Morto (no. 2) was found an association consisting of *Polygonum maritimum*, *Salsola Kali Tragus*, and a species of grass undeterminable at this season. In the immediate neighborhood occurred occasional specimens of *Aster Tripolium* and much *Plantago Coronopus*.

In the beach mud from Lake Lucrinus (no. 3) I found no member of Association A except *Inula viscosa*. This was extremely luxuriant, with branches attaining a diameter of 4^{cm} instead of the diameter of 0.8 to 1^{cm} usual in ordinary localities. The only associates identified in the very limited area examined were *Statice Limonium*, *Ficus Carica*, and *Inula crithmoides* (?).

It is evident from the analyses given that none of the soils examined were saline in any such degree as the familiar salt marshes of the

[2] Roth, Justus, Allgemeine und Chemische Geologie 1:524. 1879.

New England coast and the maritime provinces of Canada.[3] I have not indeed been able to find any typical salt marshes in the Naples region, since the beaches are often for long distances walled or protected by riprap against the encroachment of the sea, and nearly land-locked bodies of salt water such as Lake Lucrinus and the Mare Morto are surrounded by vertical stone walls, to admit of the utilization of the adjacent land to the water's edge.

It would *a priori* seem probable that the plants of Association A would belong to the category of littoral species rather than of genuine halophytes, and the results of my cultures confirm this supposition.

It seemed to the writer that the questions most worthy of investigation in connection with the association studied were: (1) the relative sensitiveness of the species to the effect of sea water or sodium chlorid solutions;[4] (2) the relative sensitiveness of strand-grown and inland-grown specimens of the same species to the effect of such solutions.

Thrifty self-sown seedlings of the species of Euphorbia, Matthiola, and Senecio in the list of members of Association A were found in great abundance, and therefore special attention was given to these species, though larger plants of all the others which could be obtained in good condition were also studied. Sea water and pure sodium chlorid, in solutions ranging from 1 to 6 per cent. of the salt were used for the cultures. The roots of the plants were partially freed from adhering sand or earth by careful immersion in a solution of the same strength as that employed in the culture, and then the plants were transferred to small tumblers containing the culture solution in which the roots were immersed. Each tumbler was covered by a disk of waterproofed pasteboard, fitted around the protruding stems of the seedlings, sufficiently close to prevent much evaporation, but not to hinder aeration of the solution. The room temperature during the two and a half months devoted to the investigation usually ranged between 12° or 15° C. by night and 20° or 22° by day, and the plants were about 1.5^{m} in front of a south window 2.5^{m} wide.

3 See GANONG, W. F., The vegetation of the Bay of Fundy salt and diked marshes. Bot. Gaz. **36**: 286, 292. 1903.

4 The writer took for granted the accuracy of H. COUPIN's statement that the fatal effect of sea water upon vegetation is due to its sodium chlorid. See his article: Sur la toxicité de chlorure de sodium, etc. Rev. Gén. Botanique **10**: 177. 1898.

In order to avoid the possible presence of traces of salts of copper or other injurious metals sometimes found in distilled water the solutions were made up with very pure cistern water.

Much difficulty was experienced in getting perfectly comparable results from the fact that slight individual differences in the plants (such as relative development of the root system) made decided differences in their tolerance of the saline solutions employed. Many values were discarded, from the fact that they were evidently erroneous from variations of this kind. In general, as was to be expected, the duration of life in sea water was considerably greater than in a pure sodium chlorid solution containing the same per cent. of this salt (2.88) that is found in Mediterranean sea water. Some of the principal results obtained are summarized in the following table:

ORDER OF RESISTANCE TO SALINE SOLUTIONS.

	In 2.88 per cent. solution NaCl, lived days	In 100 per cent. sea water, lived days
Euphorbia Paralias	17	20
Matthiola sinuata	16	19
Euphorbia terracina	11–13	9
Echinophora spinosa	15	7
Polygonum maritimum	9–10	
Alyssum maritimum (littoral)	8	7
" " (inland)		7
Senecio vulgaris (littoral)	3	5
" " (inland)		5

It is noteworthy that the littoral and the inland specimens of Alyssum and of Senecio were equal in their resisting power, as might have been expected from the comparatively equal amounts of chlorid in the beach sand and the vineyard soil.

Some experiments were made to ascertain the maximum per cent. of seawater which could be tolerated by the species of Association A without speedy appearance of symptoms of injury, such as partial drying up or death of shoots. Difficulty was found in discriminating between retardation of growth and weakening caused by culture under artificial conditions, and that due solely to the excessive salinity of the culture solutions. It was considered that a plant was unharmed if it showed no decided ill effects from the solution (except retarded

growth) after the culture had continued for a month. The results appended are only approximate.

	PER CENT. OF SEA WATER TOLERATED WITHOUT SPEEDY INJURY.
Matthiola sinuata	75
Euphorbia Paralias	63
Echinophora spinosa	50 or more
Polygonum maritimum	50
Euphorbia terracina	50 or less

For the sake of comparison a few cultures were made of species growing in full sunlight in the vineyard the soil of which was analyzed for chlorin as above given. All soon succumbed to the effect of 50 per cent. sea water (and in general still more readily to 1.5 per cent. sodium chloride solution). Classed according to the readiness with which they wilted and then died, their sensitiveness to the salts in solution was about in the following order.:

1. Lamium amplexicaule.
2. Fumaria Gussonii.
3. Papaver Rhoeas.
4. Polygonum aviculare monspeliensis.
5. Veronica Tournefortii.
6. Geranium molle.
7. Rumex bucephalophorus.
8. Euphorbia Peplus.
9. Anthemis arvensis var.

Roughly speaking, the order above given is that in which these annuals wither and die at the onset of the summer drought, except as regards no. 3, which persists well into the dry season.

In order to compare the behavior of the plants of Association A with that of true halophytes, cultivated in saline solutions, some seedlings of *Salsola Kali Tragus* were procured from the sand adjacent to Mare Morto, above described. The plants were 2–2.5^{cm} high and cultures were made of these in clean sand, rinsed with the saline solution to be used, and then flooded once a day with another portion of the solution, which was afterwards poured off. The concentrations employed were respectively of 4, 5, 5.5, 6, and 7 per cent. of sodium chlorid, and other specimens were cultivated in solutions without sand.

In sand the plants appeared normal up to and including 6 per cent. sodium chlorid solution, though growth was slow for all concentrations above 4 per cent. Without sand prompt loss of turgor was noticed in 6 per cent. solution, corresponding pretty nearly to that observed in the solution of 7 per cent. with sand. This dimin-

ished effect of the salt in the presence of sand is in accordance with the conclusions of TRUE and OGLEVEE[5] in regard to the lessened effect of toxic substances in aqueous solution in presence of inert and insoluble solids.

None of the work done was shaped with special reference to determining whether the injurious action of saline solutions on the plants examined was due to dehydrating or other physical effects, or whether it was purely toxic. A notable desiccation and shrinkage was often observed throughout the stem while the upper leaves and especially the terminal bud remained vigorous. This fact would tend to confirm the hypothesis that the lethal action of dissolved salts is of a physical character. On the other hand, the fact that a pure sodium chlorid solution is usually more quickly fatal than sea water containing the same amount of sodium chlorid plus other salts tends to confirm the toxic hypothesis. Probably both factors may cooperate to produce death. Apparently the relative ease with which root hairs of the species studied can be plasmolyzed does not bear any definite relation to the susceptibility of the species to the action of sodium chlorid solutions.

The principal conclusions reached may be summarized as follows:

1. Association A consists of members very unequally resistant to the action of pure sodium chlorid solutions and of sea water.

2. The tolerance of sodium chlorid on the part of some members of this association is considerably greater than that of ordinary non-littoral plants; in other words, they are facultative halophytes.

3. Many of the species of this association are typical psammophytes, none are typical halophytes.

4. Growth of a non-halophytic species for many generations in an atmosphere at times highly charged with saline spray does not bring about greater tolerance of saline solutions when absorbed by the roots.

5. The Salsola seedlings studied showed a tolerance of sodium chlorid solutions up to almost 6 per cent. as good as that of any member of Association A for the 2.88 per cent. solution.

NAPLES, ITALY.

[5] TRUE R. H., and OGLEVEE, C. S., The effect of the presence of insoluble substances on the toxic action of poisons. Bot. GAZETTE 39:1–21. 1905.

NEW AND NOTEWORTHY NORTH AMERICAN SPECIES OF TRIFOLIUM.

HOMER DOLIVER HOUSE.

(WITH TWELVE FIGURES)

I. NEW OR NOTEWORTHY SPECIES OF THE UNITED STATES.

THE following notes upon the genus Trifolium are based upon material in the National Herbarium. All figures × 1½.

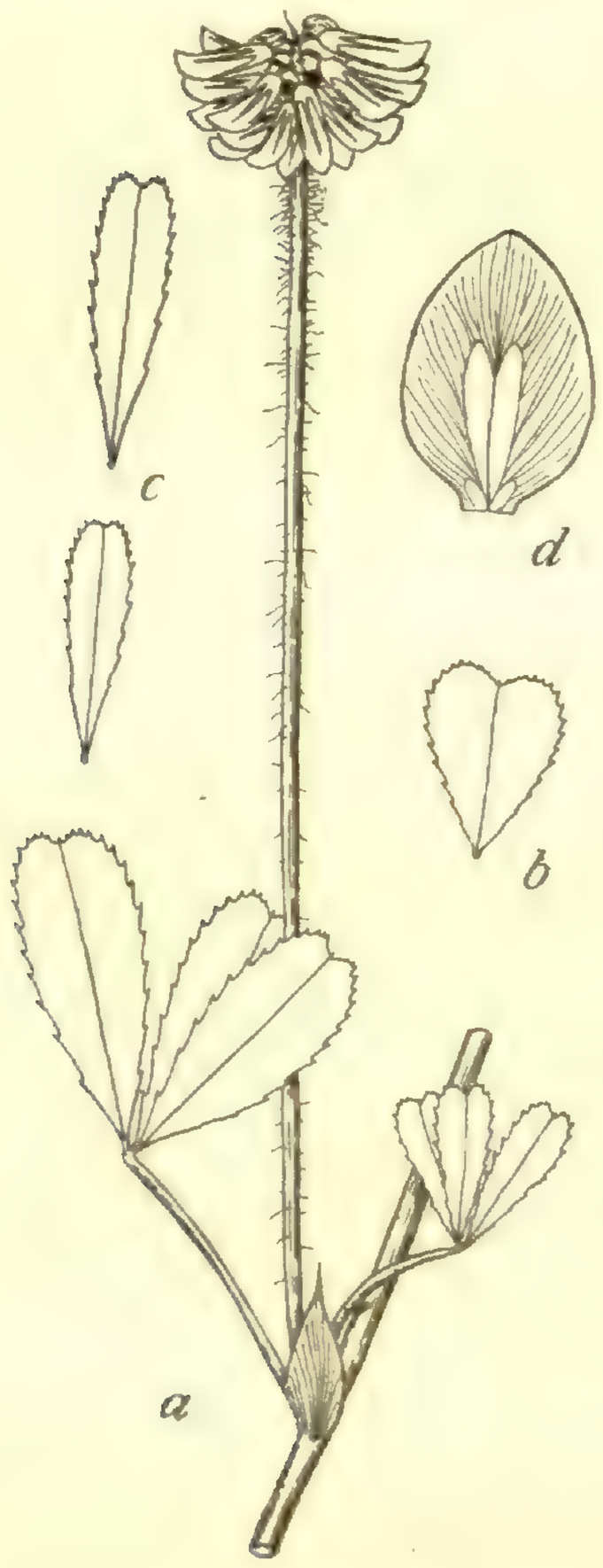

FIG. 1.—*Trifolium Greenei* House: *a*, portion of type specimen; *b*, leaflet from lower leaf; *c*, leaflets from upper leaves; *d*, banner.

Trifolium Greenei, nom. nov.—(*Fig. 1*). —*T. bifidum decipiens* Greene, Fl. Fran. 24. 1891; not *T. decipiens* Hornem., Hort. Hafn. **2**:715. 1815.—Of much broader distribution and apparently not merging into *T. bifidum* Gray, though closely related to that species. It has more of the general appearance of *T. gracilentum* T. & G., but distinguished from it by its villous-pubescent peduncles and cuneate-oblong, subglaucous, and retuse leaflets.

Low moist places or natural grassy meadows, from San Diego to Mendocino and Butte Counties, California. The type, collected at Berkeley by *Greene*, is in the herbarium of Professor Greene.

TRIFOLIUN BIFIDUM Gray (*fig. 2*) seems to be a species peculiar to the bay region of California only; the type, collected by *Brewer* (no. 1184, 1862), "near Marsh's Ranch, between Monte Diablo and the San Joaquin (Contra Costa Co.), among grass in a ravine near the water, May 29," is in the National Herbarium.

TRIFOLIUM BREWERI S. Wats., Proc. Am. Acad. **11**:131. 1876.—*T. amabile* Loja., Nuovo Giorn. Bot. **15**:142. 1883, ex descr.

A careful reading of LOJACONO'S paper on North American species of Trifolium makes very apparent the fact that he had a very scanty representation of the forms and distribution of our western species. While his descriptions are excellent, he has failed in many instances to determine properly the types of the species.

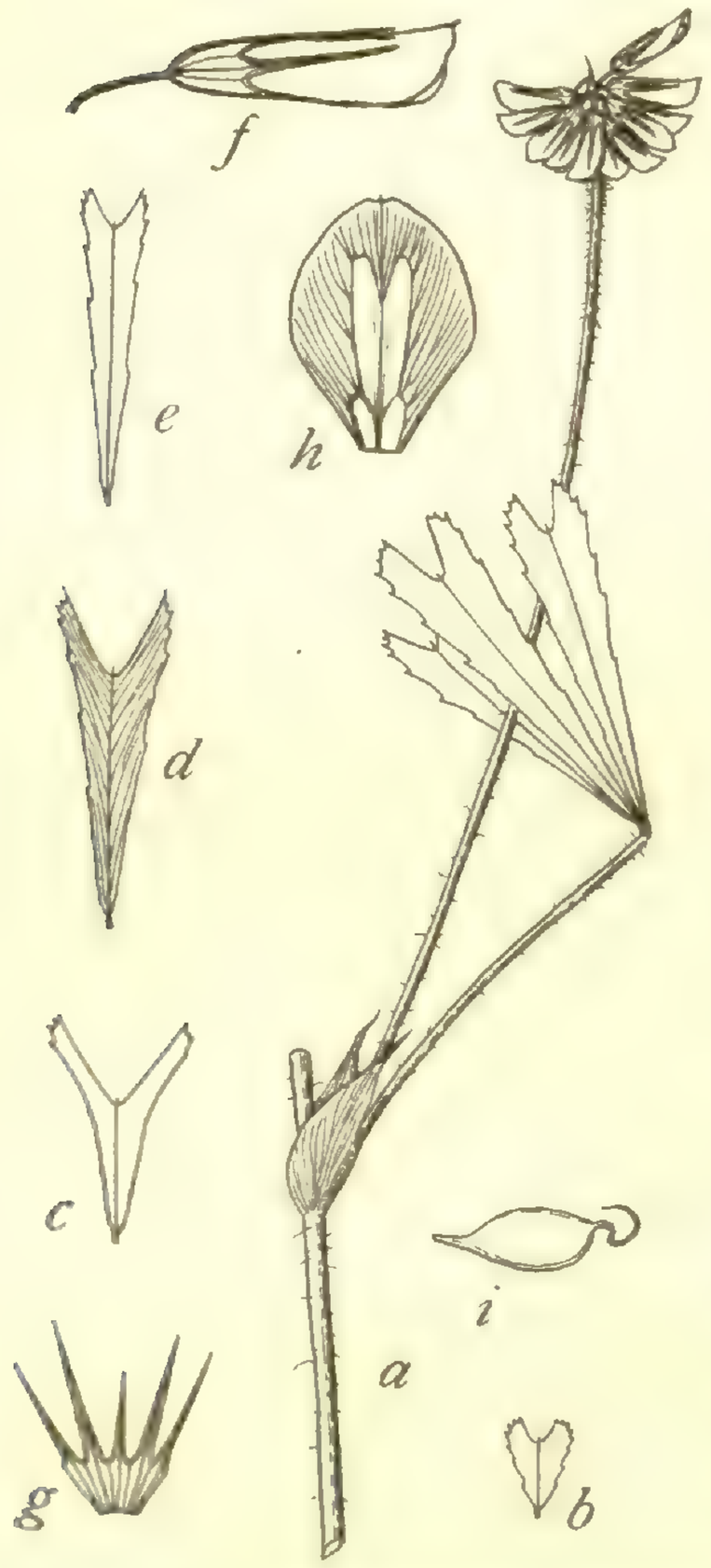

FIG. 2.—*Trifolium bifidum* A. Gray: *a*, portion of type specimen; *b* and *c*, leaflets from lower leaves; *d* and *e*, leaflets from upper leaves; *f*, flower; *g*, calyx expanded; *h*, banner; *i*, legume.

Trifolium Douglasii, nom. nov.—*T. altissimum* Dougl., Hook. Fl. Bor.-Am. 1:130. *pl. 48.*, 1830; not *T. altissimum* Lois. 1807.

An abundant clover in moist or natural wet meadows of eastern Washington, Oregon, and adjacent Idaho. Flowering in June and July, fruiting from July to the end of August.

TRIFOLIUM HARNEYENSE Howell, Fl. Northwest Am. 134. 1898.—*T. arcuatum Cusickii* Piper, Bull. Torr. Bot. Club 29:642. 1902.

An examination of the floral parts of the available herbarium material of *T. Harneyense* and *T. arcuatum Cusickii* shows them to be identical.

Trifolium villiferum, sp. nov.—*Fig. 3.*—Related to *T. eriocephalum* Nutt. Stems slender, erect, from perennial, ascending, and branching roots, 25 to 35 cm high, densely villous-pubescent with long hairs, stem nearly glabrous at base only: leaflets oblong-lanceolate, 2.5 to 4 cm long, 6 to 13 mm wide, obtuse at base, usually acute at apex, irregularly and inconspicuously repand-denticulate, pale green, appearing almost glaucous by the dense, whitish indument, scarcely less

FIG. 3.—*Trifolium villiferum* House: *a*, flower; *b*, calyx expanded; *c*, banner, wing, and keel.

pubescent above than below; petioles 3 to 7^{cm} long, uppermost shortest; stipules lanceolate or the upper ovate-lanceolate, subfoliaceous, 2 to 3.5^{cm} long, acuminate, entire or sparingly toothed: inflorescence pseudo-terminal; peduncles 6 to 13^{cm} long; heads densely many-flowered, ovoid when young, flowers all becoming strongly reflexed: calyx densely villous with shaggy hairs without especially toward the apex, tube about 2^{mm} long, the 5 subulate nearly equal teeth plumose, 3 to 4^{mm} long, somewhat bent in age: corolla pinkish-purple, 12 to 14^{mm} long; banner oblong, rounded or obscurely retuse at apex, broadest (about 6^{mm}) below the middle; wings shorter, tapering to a blunt apex, blade with a strong basal auricle; keel still shorter and more acute: legume ovoid, sessile, densely plumose-pubescent, pubescence extending nearly to tip of style, 2-seeded.

Flowering in June and July, fruiting in July and August. Southern Utah, *Palmer* (no. 91), 1877 (type in the U. S. National Herbarium); Burrville, Sevier Co., *Jones* [(no. 5642a), July 17, 1894, 2100^{m} alt.; Deep Creek, *Jones*, June 6, 1891.

FIG. 4.—*Trifolium shastense* House.

Trifolium atrorubens (Greene), comb. nov. —*T. Rusbyi atrorubens* Greene, Erythea **4**:66. 1896.

[Type, collected by *Parish* (no. 3745), Buff Lake, San Bernardino Co., California, June 21–27, 1895, in the herbarium of Professor Greene.

[Examination of the type and several other sheets of *T. atrorubens* from southern California shows that it is not nearly so closely related to *T. Rusbyi* as to *T. pedunculatum* Rydb., and should properly be given specific rank.

Trifolium shastense, sp. nov.—*Fig. 4.*—Related to *T. longipes* and *T. oreganum*. Stems numerous from matted, branching, and slender rootstocks (forming a sod), 10 to 15^{cm} high; silky pubescent above and beneath on the leaves; stems purplish below: stipules lanceolate, green, aristate-acuminate, entire or few-toothed, 12 to 20^{mm} long; leaflets lanceolate, acute at base, often broadest above the middle, apex aristate-acuminate, margins prominently spinulose-

denticulate, glabrous above, silky-pubescent beneath, 15 to 25^{mm} long, 4 to 7^{mm} wide; leaflets of the lower leaves relatively broader and shorter, nearly obovate-cuneate, acute or rounded; petioles mostly shorter than leaflets, but lower ones longer: inflorescence usually solitary; peduncles 5 to 8^{cm} long, somewhat woolly-pubescent above with whitish hairs, densely many-flowered in a globose head; flowers sessile, the outermost spreading or becoming reflexed, 10 to 13^{mm} long: calyx silky-pubescent or becoming glabrate, tube 1.5 to 2.5^{mm} long, the five filiform-subulate teeth straight, 8 to 10^{mm} long, upper ones shortest and scarcely more than 8^{mm} long, sometimes shorter: banner sublanceolate, broadest (about 5^{mm}) below the middle, acuminate-pointed at the apex; wings and keel shorter, wings conspicuously attenuate-pointed, keel acute: legume stipitate, 2-seeded.

North side of Mt. Shasta, Siskiyou Co., California, 1500–2700^{m} alt. Collected by *H. E. Brown* (no. 362), type in the U. S. National Herbarium, June 11–16, 1897. No. 365 of the same collection is identical.

Remarkable for its sharply serrated and pointed leaflets, acuminate-pointed banner, and attenuate-pointed wings.

Trifolium Covillei, sp. nov.—*Fig. 5.*—Related to *T. latifolium*. Stems very short and leafy, several from a solitary, perpendicular thickened perennial root and appearing as a dense green mat at its apex, the root 10 to 20^{cm} deep; stems 1 to 3^{cm} long: stipules small, ovate, 5 to 7^{mm} long, blunt and often rounded at apex, entire, adnate to the petiole for two-thirds their length; leaflets obovate-subcuneate, rounded or retuse at apex, rarely the upper acute, margins finely but not sharply or conspicuouly toothed, 6 to 12 ^{mm} long, silky pubescent beneath, glabrous above; petioles mostly shorter or but little longer than the leaflets: peduncles 8 to 10^{cm} long, much exceeding the leafy part of the plant, silky-pubescent above; heads globose, 40 to 60-flowered, 2 to 2.5^{cm} in diameter, some of the flowers spreading or becoming reflexed,

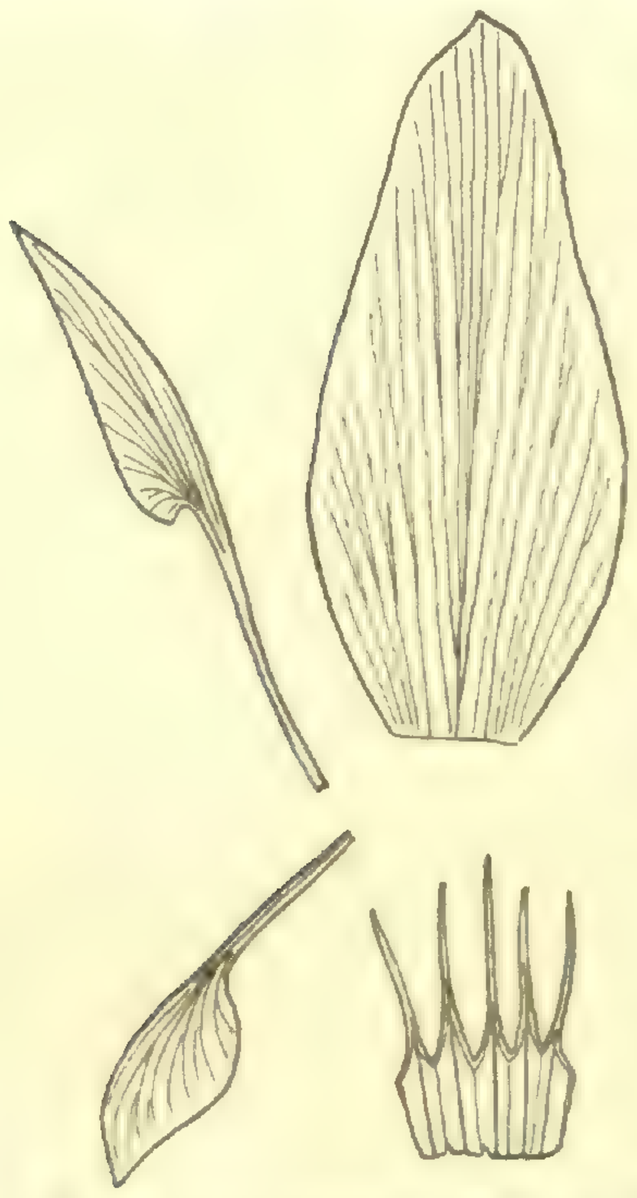

FIG. 5.—*Trifolium Covillei* House.

all sub-sessile: calyx membranaceous, the tube 1.5 to 2^{mm} long, sparingly hairy above, the 5 filiform-subulate teeth subequal, 2.5 to 3.5^{mm} long: banner yellowish, 12 to 14^{mm} long, inflated and including the wings and keel, broadest (6 to 7^{mm}) below the middle, acute, wings subacuminate, keel acute: legume short-stipitate, 2-seeded.

Bog-lands in the Wenatchee Mountains, Kittitas Co., Washington, *Coville* (no. 1180), Sept. 4, 1901 (type in the U. S. National Herbarium).

The group of small species related to *T. monanthum* Gray has been not a little confused by various authors, and the location of the type in the U. S. National Herbarium makes it possible to define definitely its critical parts. The accompanying description and drawing are from the type.

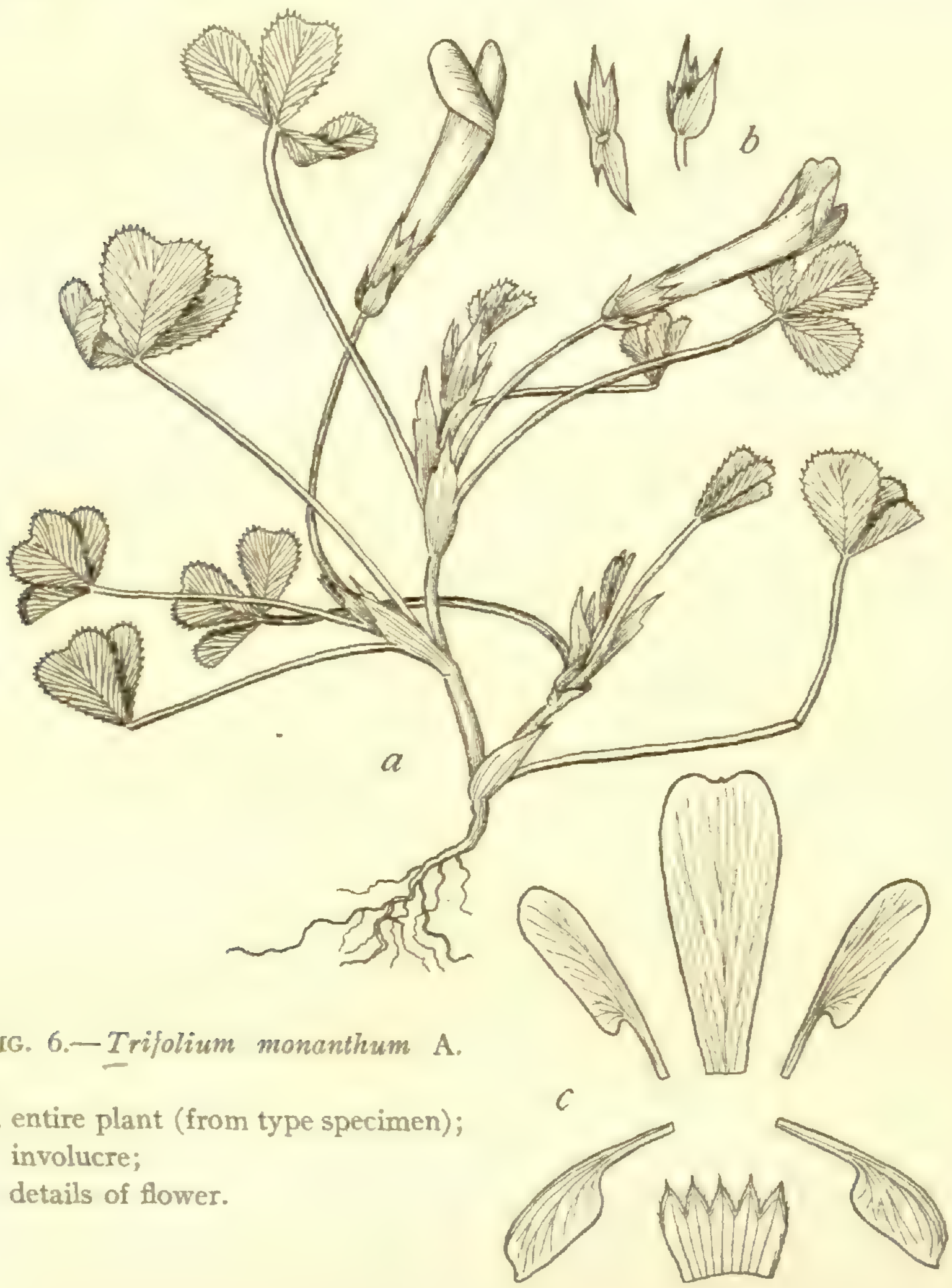

FIG. 6.—*Trifolium monanthum* A. Gray:

a, entire plant (from type specimen);
b, involucre;
c, details of flower.

TRIFOLIUM MONANTHUM A. Gray, Proc. Am. Acad. **6**:523. 1865.—*Fig. 6.*—Perennial from numerous, slender, branching roots; stems branching from near the base, ascending, 2 to 4^{cm} high, only sparingly villous: stipules ovate-lanceolate, green, 3 to 5-nerved, cuspidate-acuminate, 2 to 4^{mm} long, entire or rarely with a few minute rigid teeth near the apex; leaflets obovate-cuneate, retuse or truncate at apex, margins spinulose-denticulate, 4 to 6^{mm} long, sessile, pale green with a few villous hairs beneath, darker green above, the ascending, branching whitish veins ending in the subcallous mucronate teeth of the margin; petioles filiform, longer than the leaflets: peduncles filiform, mostly shorter than the leaves, one-flowered (rarely 2), involucre of two entire or sparingly toothed, ovate-lanceolate, cuspidate-acuminate bracts, 2 to 3^{mm} long: calyx cylindrical, twice as long as involucre, about 4^{mm} long, sparingly villous, 10-nerved, 5 of the nerves more prominent than the others, the triangular-lanceolate, spinulose-acuminate teeth about one-third the length of the tube: corolla 9 to 12^{mm} long, purplish-white, the slender tube elongated and somewhat glandular, not scarious or inflated after flowering; banner lanceolate-obovate, retuse; wings shorter and rounded; keel sub-acute: legume stipitate, 2-seeded.

Flowering from the middle of June to September; fruiting from July to October.

Mountain meadows, banks, and grassy places, Sierra Nevada in California from Alpine to Tulare County, and in Ormsby County, Nevada; 2100 to 3000^{m} alt.

CALIFORNIA: Sierra Nevada, *Lemmon* 1875; Manachi Meadows, 2500^{m} alt., *Rothrock* (no. 307), Sept. 1875; Tuolumne Co.: "Moist bank by Soda Springs, alt. 2650^{m}," *Brewer*, June 26, 1863 (no. 1704), type in U. S. National Herbarium; vicinity of Tuolumne Meadows, 2600–2900^{m} alt., *Hall* and *Babcock*, July 1902 (no. 3625); Alpine Co.: Caple's Lakes, 2600^{m} alt., *Hansen*, June 21, 1892 (no. 351); Fresno Co.: meadows near Block mountain, 3000^{m} alt., *Hall and Chandler*, July 1900 (no. 613); Tulare Co. near Mineral King, 2750^{m} alt., *Coville and Funston*, Aug. 4, 1891 (no. 1473).

NEVADA: Ormsby Co.: Snow Valley, 2460–2615^{m} alt., *C. F. Baker*, July 8, 1902 (no. 1282).

TRIFOLIUM TENERUM Eastw., Bull. Torr. Bot. Club **29**:81. 1902. "Higher meadows on the trail to the South Fork of King's River (Fresno Co.), California. It was collected by the writer at Summit and Bearskin Meadows, July 1–13, 1899. The first named speci-

mens are considered the type specimens. Type in the Herbarium of the California Academy of Sciences."

Characterized by the canescent and softly villous foliage, strongly nerved and setosely serrulate leaflets; heads 1 to 6-flowered; involucre glabrous, of 2-5 separate, laciniate-aristate bracts, 2 to 4 mm long; banner of the corolla with three rounded teeth at the truncate apex; wings slender, as long as the banner, auriculate at base of blade; keel two-thirds as long, tipped with an obtuse erect beak, the keel itself purple, auricled at base; ovary obovate, pilose at summit, one-ovuled.

TRIFOLIUM GRANTIANUM Heller, Muhlenbergia 1:136.

Undoubtedly distinct from *T. tenerum* Eastw., as HELLER indicates, but it is unfortunate that the floral characters were not better described ["Calyx cylindrical, or somewhat campanulate, 4 mm long, the tube 2 mm long, more or less membranous, veins prominent; the narrowly lanceolate teeth aristate, green: corollas 1 cm long, slender, 2 mm across, whitish, the hood of the keel purple"] when it is considered that in this group of small species the floral characters are of the utmost importance.

Based on *Grant's* Number 6343, July 23, 1904, from San Bernardino Co., Calif.

TRIFOLIUM PARVUM (Kellogg) Heller, Muhlenbergia 1:114. 1905.—*Fig. 7.*—*T. pauciflorum* (?) var. *parvum* Kellogg, Proc. Cal. Acad. **5**:54. 1873; *T. multicaule* Jones, Bull. Torr. Bot. Club **9**:31. 1882.—The prostrate or slightly ascending stems 10 to 20 cm long, often many from a thickened root; softly silky-pubescent, sometimes densely so: leaflets obovate, retuse or obtuse or sometimes those of upper leaves short acute at apex, subcuneate at base, minutely spinulose-serrulate; stipules broadly ovate, sharply toothed and acute; petioles filiform, the lower 1 to 3 times the length of the leaflets, the upper scarcely longer than the leaflets: peduncles exceeding the leaves, 1 to 3 cm long, 1 to 7 (usually 2 to 5)-flowered; involucre 5 to 7-divided into triangular-lanceolate acute and entire segments, 1 to 2 mm long, these spreading in age, rarely somewhat toothed: calyx 3 to 4 mm long, villous-pubescent, the 5 triangular lanceolate teeth about equaling the tube in length, the lower one

a little shorter and broader than the others, all spinulose-acuminate: corolla a little more than twice the length of the calyx, white with a purple-tipped keel; banner narrowly oblong, about 10^{mm} long and 3.5 to 4.5^{mm} wide, deeply retuse at apex; wings and keel much shorter, the keel acute: legume 2-seeded.

Around springs and in natural moist meadows of the Sierra Nevada, California, from Nevada Co. to Acador Co., Yosemite Park, and Fresno Co., at 1500 to 2100^{m} alt.

Nevada Co.: Soda Springs, *Jones* (no. 2592), July 30, 1881 (type of *T. multicaule*, in U. S. National Herbarium); low ground on south side of Donner Lake, *Heller* (no. 6942), July 16, 1903.

Amador Co.: Bear River, *Hansen* (no. 1968), Aug. 30, 1896.

Yosemite Park: Hetch-Hetchy Trail, *Hall* and *Babcock* (no. 3385), July 1902.

Fresno Co.: Pine Ridge. *Hall* and *Chandler* (no. 256), June 15–25, 1900.

Trifolium simulans, sp. nov.—*Fig. 8.*—Perennial from slender, branching roots; stems numerous, prostrate or ascending, slender, 5 to 20^{cm} long, glabrous: stipules ovate-lanceolate, 6 to 10^{mm} long, green or the lower ones subscarious, spinulose-acuminate, and with a few aristate teeth, 5 to 7-nerved; leaflets oblong-lanceolate, the middle one and often the others cuneate, irregularly spinulose-denticulate, mucronate-tipped at the acute apex, 9 to 14^{mm} long, 2.5 to 5^{mm} wide; petioles filiform, 2 to 4 times the length of the leaflets: peduncles as slender and shorter than the petioles; involucre divided into 2 or 3 parts, these cleft again to make 5 to 7 narrow, spinulose-acuminate, conspicuously nerved, simple or few-toothed segments to the entire involucre: calyx short-pedicelled, glabrous, about twice the length of the involucre, teeth subulate-acuminate, 3 to 4^{mm} long, the lower slightly shorter: corolla very large for the size of the plant, white with a purple-tipped keel; banner narrowly oblong, about 15^{mm} long, 5^{mm} wide, retuse and mucronate at apex; wings

FIG. 8.—*Trifolium simulans* House.

and keel much shorter, blade of wings about 9^{mm} long, that of the acute keel only about 4^{mm} long: legume 2-seeded, sessile.

San Jacinto Mountains, California, 1800 to 2700^{m} alt., *H. M. Hall* (no. 710), July 22, 1897, type in the U. S. National Herbarium); Strawberry Creek (San Jacinto Mts.) 1600^{m} alt., *H. M. Hall* (no. 2200), June 20, 1901.

Resembling *T. parvum* in size, but very distinct from it in the remarkably large flowers for the size of the plant, the leaf, calyx, and corolla characters also showing well-marked differences. It appears to be as distinct from *T. parvum* as *T. tenerum* is from *T. monanthum*, and to show these differences descriptions of all three species are given.

II. MEXICAN SPECIES.

TRIFOLIUM AMABILE HBK., Nov. Sp. & Gen. **6**:503. *pl. 593.* 1823; *T. Humboldtii* Spreng., Syst. **3**:313. 1826 (*T. pauciflorum* Willd. herb.); *T. Hemsleyi* Loja., Nuovo Giorn. Bot. **15**:143. *pl. 4. fig. 1.* 1883.

One of the commonest species of Mexico and distributed from northern Mexico to Central America.

TRIFOLIUM GRACILENTUM T. & G., Fl. N. Am. **1**:316. 1838; *T. denudatum* Nutt., Proc. Acad. Phila. II. **1**:152. 1848.

Lower California, San Quentin Bay, *Palmer* (no. 613), Jan. 1889.

Trifolium longifolium (Hemsley), comb. nov.—*T. amabile* var. *longifolium* Hemsley, Biol. Cent. Am. Bot. **1**:232.1879; *T. goniocarpum* Loja., Nuovo Giorn. Bot. **15**:145. *pl. 4. fig. 2.* 1883.

HEMSLEY'S description is based upon *Parry* and *Palmer's* no. 134, although other specimens are mentioned. LOJACONO'S description is also based upon a plant collected by *Parry* and *Palmer*, but no collection number is given. His description, however, agrees well with a duplicate of HEMSLEY'S type in the National Herbarium, and the conclusion was forced upon me that they are identical.

San Luis Potosi, *Parry* and *Palmer* (no. 134), 1878: Chihuahua, *Pringle* (no. 1208), 1887; *Townsend* and *Barber* (no. 177), 1899; *Palmer* (no. 385), 1885: Durango, *Palmer* (no. 237), 1896: Tepic, *Rose*, Aug. 9, 1897: Jalisco, *Palmer* (no. 236), 1886: Aguascalientes, *Rose* and *Painter* (no. 7795), 1903: Federal Dist., *Pringle* (no. 7492), 1897: Vera Cruz, Orizaba, *Bourgeau*, 1865–66; *Seaton* (no. 93), 1891: Oaxaca, *Rose* and *Hough* (no. 4644), 1899.

Trifolium Lozani, sp. nov.—*Fig. 9.*—Related to *T. mexicanum.* Stems numerous, spreading and ascending from a perennial root densely silky-pubescent, 10 to 20^{cm} long; the internodes relatively

short: stipules ovate, lower scarious and entire, upper green and sparingly toothed, all acuminate, 6 to 10^{mm} long, 4 to 6^{mm} wide; leaflets cuneate-obovate or cuneate-oblong, sessile, 8 to 15^{mm} long, 5 to 10^{mm} wide, rounded at apex, glabrous above, sparingly pubescent beneath, subentire, the minute teeth very inconspicuous; petioles mostly shorter than the leaflets, or the lower slightly longer: peduncles scarcely exceeding the leaves, 1 to 3^{cm} long; heads globose, ebracteate, 25 to 50-flowered; flowers short-pedicelled, all becoming reflexed: calyx tube subcampanulate, pubescent, slightly more than 1^{mm} long; the subulate teeth twice as long, four of them ascending, lower one straight: corolla yellowish; banner broadly oblong, 5^{mm} long or less, retuse; wings and keel much shorter, subacute: legume oblong in outline, very blunt at apex, 3^{mm} long or less, 4-seeded: seeds nearly as thick and broad as long, smooth and brownish, less than 1^{mm} long.

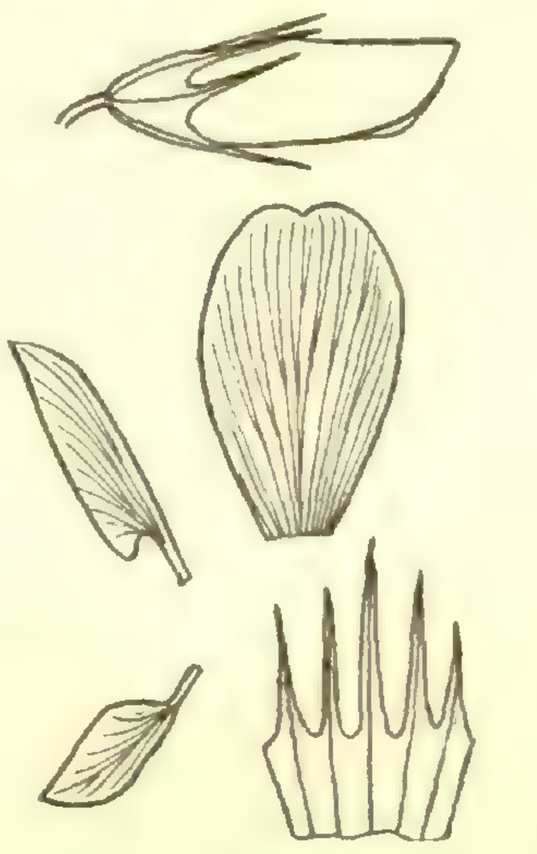

FIG. 9.—*Trifolium Lozani* House.

Eslava, Federal District, 2300^{m} alt., *Pringle* (no. 9512), June 15, 1901 (type sheet no. 396298 in U. S. National Herbarium). Distributed as *T. amabile*, which it in no way resembles. It differs from *T. mexicanum* by its more densely pubescent stems and peduncles, smaller flowers, and relatively shorter calyx teeth. Named in honor of Señor Filemon LOZANO, assistant to Mr. PRINGLE.

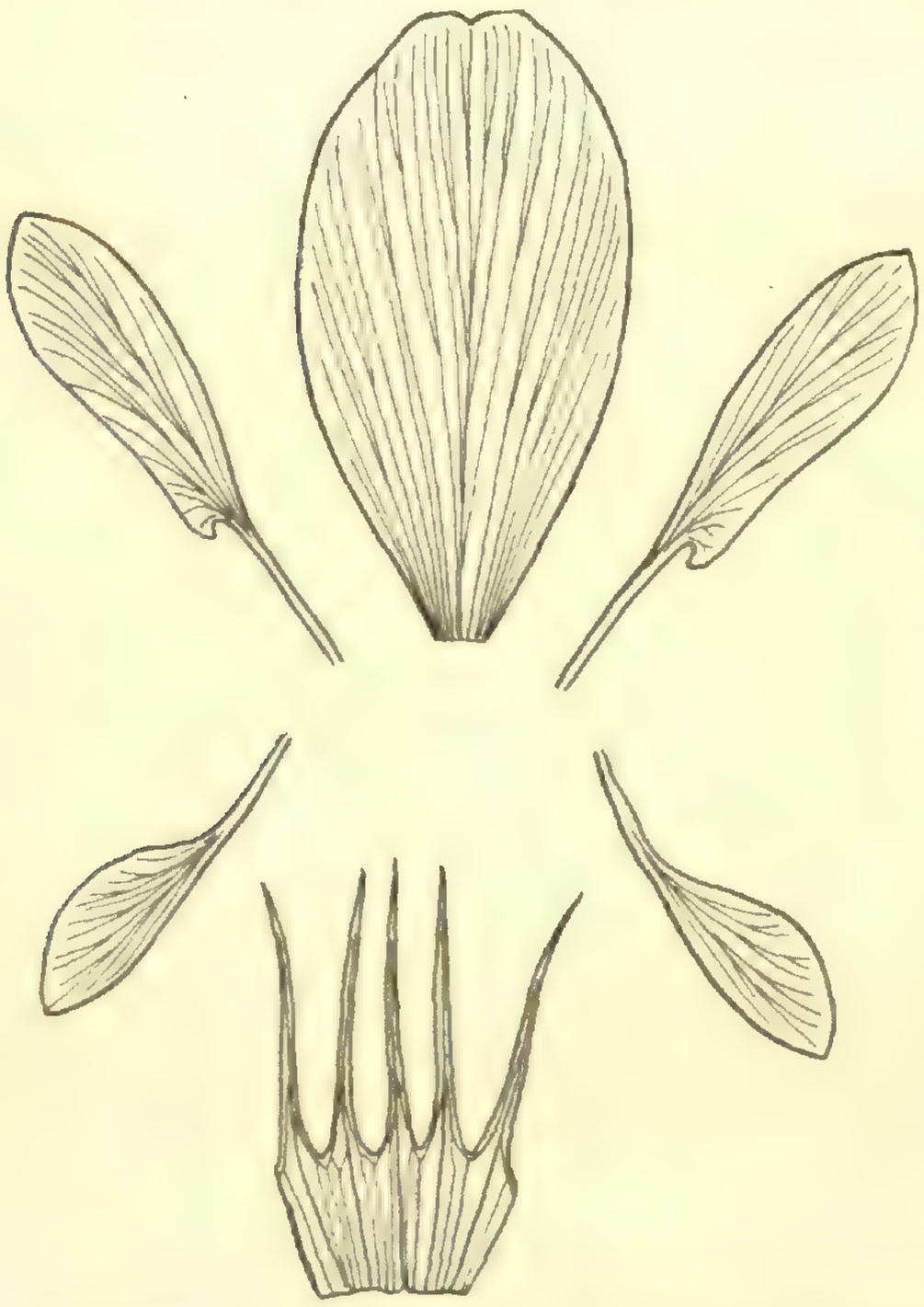

FIG. 10.—*Trifolium mexicanum* Hemsley.

TRIFOLIUM MEXICANUM Hemsley, Biol. Centr. Am. Bot. **1**:233. 1879.—*Fig. 10.*—*T. potosanum* Loja., Nuovo. Giorn. Bot. **15**: 144. *pl. 2.* 1883.

The type of *T. mexicanum* is from San Luis Potosi (*Parry* and *Palmer* no. 137, 1878), and upon the same number

is based *T. potosanum* Loja. A duplicate type is in the U. S. National Herbarium.

The following specimens from central Mexico differ from typical *T. mexicanum* in having larger flowers, more pubescent stems, and blunter leaflets, and are more spreading in habit. They may represent a variety, but scarcely more.

Mexico (state): hills near Ozumba, 2400^{m} alt., *Pringle* (no. 9775), Nov. 8, 1902; Flor de Maria, *Pringle* (no. 3238), Sept. 4, 1890; *Rose* and *Painter* (no. 7816), Oct. 13, 1903.

Trifolium Nelsoni, sp. nov.—*Fig.* 11.—Related somewhat to *T. mexicanum* but scarcely resembling it. Stems spreading and ascending from a perennial root, minutely pubescent, about 50^{cm} long or less: stipules ovate-lanceolate, green, rigid and the lower scarious, aristate-acuminate, entire, 15 to 20^{mm} long; leaflets ovate-lanceolate to elliptic-oblong, sparingly pubescent and pale beneath, green and glabrous above, usually with a whitish V-shaped blotch on the upper surface, apex acute or blunt, callous-tipped, margins inconspicuously repand-denticulate; petioles of the lower leaves 2 to 4 times the length of the leaflets: peduncles densely woolly-pubescent toward the summit, often 15 to 20^{cm} long; heads depressed-globose, 20 to 40-flowered, flowers subtended by subulate filiform bracts, 3 to 5^{mm} long; pedicels none or very short: calyx-tube 10-nerved, about 1.5^{mm} long, the subulate green teeth 4.5 to 5^{mm} long, subequal: corolla yellowish; banner 8 to 10^{mm} long, orbicular-obovate, slightly retuse at the apex or rounded; wings and keel shorter but relatively broad.

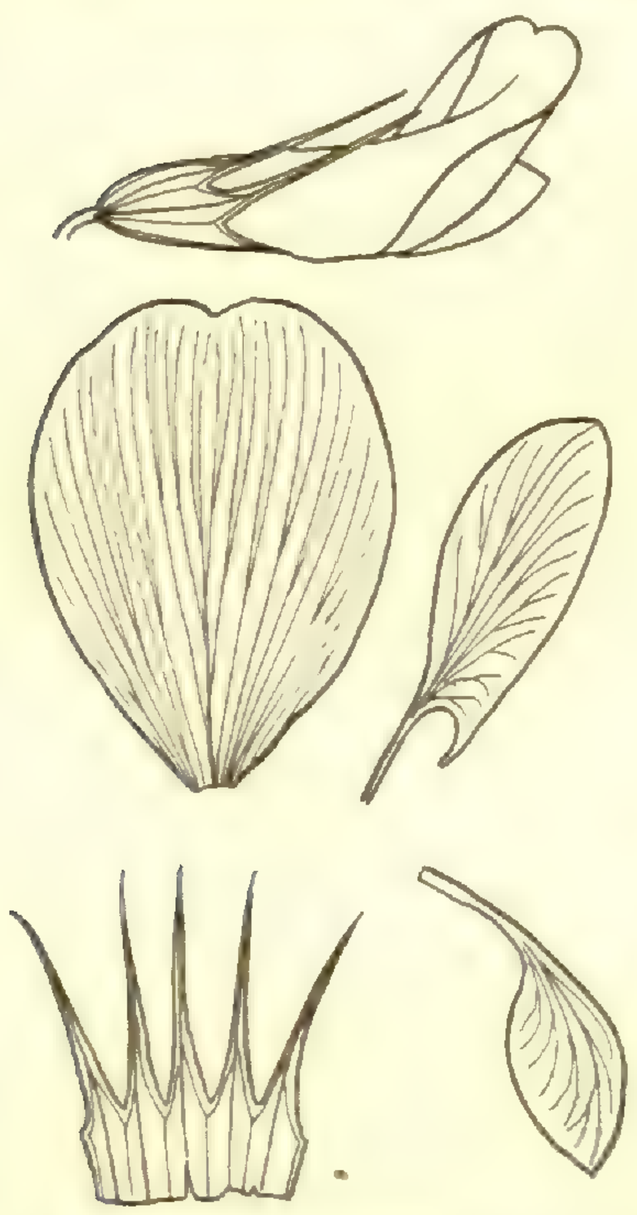

FIG. 11.—*Trifolium Nelsoni* House.

Vicinity of La Parada, Oaxaca, *E. W. Nelson* (no. 1016), Aug. 19, 1894 (type in the U. S. National Herbarium). Remarkable for the extremely broad petals.

TRIFOLIUM PALMERI S. Wats., Proc. Am. Acad. **11**:132. 1876.

Guadalupe Island: *Palmer* (no. 26, 1875), type in Gray Herbarium; a duplicate type in herbaria of Professor Greene and Columbia University; *Greene* Apr. 21, 1885; *Palmer* (no. 859), 1889; Dr. *F. Franceschi*, 1893.

Trifolium cognatum, sp. nov.—*Fig. 12.*—Related to *T. longifolium*. Minutely pubescent or glabrate; stems spreading or ascending from a thickened perennial root, 10 to 30^{cm} high, often somewhat tinged with dull purple below, striate: stipules ovate or the lower ovate-lanceolate, green, acuminate, entire and scarious margined, 12 to 16^{mm} long; leaflets elliptical-oblong, subcuneate at base, glabrous, rounded and minutely mucronate at apex, 8 to 20^{mm} long, 4 to 10^{mm} wide, margins minutely and irregularly denticulate; petioles 2 to 4 times as long as the leaflets: peduncles exceeding the leaves; heads subglobose, 15 to 40-flowered; flowers erect when young, soon becoming entirely reflexed, their pedicels 2 to 4^{mm} long: calyx with a few scattered hairs, the tube 10-nerved, about 1^{mm} long, the subequal, subulate-acuminate teeth 3 to 3·5^{mm} long: corolla 7 to 9^{mm} long; banner violet-purple, ascending, retuse at apex, nearly 5^{mm} broad; wings and keel shorter and yellowish: legume 3 or 4-seeded.

FIG. 12.—*Trifolium cognatum* House: *a*, stipule and leaflets; *b*, flower; *c*, calyx expanded; *d*, banner, wings, and keel.

Barren hills above Pachuca, Hidalgo, 2600–2900^{m} alt., *Pringle* (no. 6933), July 30, 1898. Type in U. S. National Herbarium.

Although fully matured legumes are not present on the type, the species is evidently related to *T. longifolium* (*T. goniocarpum* Loja.), from which it is distinguished by its larger flowers, slight pubescence, rounded or blunter leaflets, and more slender ovary. Peculiar in the purplish banner and yellowish wings and keel, the reverse of the condition of coloring usually seen in clovers with purple and yellow flowers.

TRIFOLIUM REPENS L., Sp. Pl. 767. 1753.

Reported by authors from the valley of Mexico and specimens from Central America have been examined. Probably introduced and escaped about many of the larger cities and seaports.

TRIFOLIUM MICROCEPHALUM Pursh, Fl. Am. Sept. **2**:478. 1814.

Guadalupe Island, *Palmer* (no. 831), Apr. 1, 1889. Probably also occurring in northern Lower California.

TRIFOLIUM RHOMBEUM S. Schauer, Linnaea **20**:740. 1847.

As I have not been able to establish fully the identity of this species, further than to advance the suggestion that it is perhaps the *T. mexicanum* of Hemsley, the original description is given here in full.

"*Trifolium* (*Trifoliastrum*) *rhombeum* S. Schauer: caulibus adscendentibus striatis cum petiolis pedunculis calycibusque villoso-lanuginosis, foliolis rhombeo-ellipticis obtusis mucronatis arguta denticulatis striato-venosis supra glabris subtus pilosiusculis glabrescentibus, stipulis membranaceis lato-ovatis mucronulatis pilosulis, capitulis axillaribus longe pedunculatis multifloris densis exinvolucratis, floribus pedicellatis demumque deflexis, calycis laciniis subaequalibus setaceis erectis tubo brevi longioribus corolla tertia brevioribus glabris.

"In montosis Mexici. Aschenb. n. 164. (perennial).

"Ex typo *Tr. hybridi* nostratis; inter mexicana forte affine *Tr. amabili* HBK., ceterum pubescentia, foliorum figura et serratura floribusque magnis insignis. Petioli 6–9 lin. longi, stipulis duplo longiores; foliola subpollicaria. Pedunculi folium longe excedentes. Flores magni, vexillo 4 lin. aequante Corolla alba vel pallide rosea ex sicco videtur, vexillum emarginatum."

TRIFOLIUM SCHIEDEANUM S. Wats., Proc. Am. Acad. **17**:338. 1883.—*T. reflexum* Schlecht., Linnaea **5**:576. 1830; not L.

"Jalapa (*Schiede*), and at Lerios, 45 miles east of Saltillo, [*E. Palmer*] (201)."

The locality for Schiede's plant as given by SCHLECHTENDAL is "Prope Jalapan ad latera montis Maculteрec, San Andres inque graminosis."

A duplicate of Palmer's plant mentioned above is in U. S. Nat. Herbarium.

TRIFOLIUM TRIDENTATUM Lindl., Bot. Reg. sub *pl. 1070*. 1827.

Lower California, Todos Santos Island, *Anthony* (no. 194), 1897; San Quentin Bay, *Palmer* (no. 697), 1889.

TRIFOLIUM WILLDENOVII Spreng., Syst. **3**:208. 1826.—*T. involucratum* Orteg., Hort. Matr. Dec. 33. 1797; Willd., Sp. Pl. **3**:1372.

1801; not *T. involucratum* Lam. 1778; *T. Ortegae* Greene, Pittonia **3**:186. 1897.

Both WILLDENOW and SPRENGEL seem uncertain regarding the native country of this species and the identification of Willdenow's name with an American species is perhaps first made by HBK. (Nov. Gen. & Sp. **6**:502. 1823): "Crescit prope Valladolid Mexicanorum alt. 1000 hex."

This species, not rare throughout northern Mexico, is not at all closely related to the species of California which for so long has passed as *T. involucratum* and which Professor GREENE has shown to be *T. Wormskjoldii* Lehm.

The important characters of *T. Willdenovii* are the linear-lanceolate stipules, subulate-acuminate and lacerate-toothed; the involucre divided nearly to the base into 6 to 8 nearly simple, subulate-aristate segments, resembling in this respect the *T. spinulosum* of northwestern United States, but not *T. Wormskjoldii* of California. The leaflets are all linear and apiculate, except those of the lower leaves which are relatively broader; the purple flowers are from 16 to 18^{mm} long, in large erect heads; the banner very narrow and retuse at apex.

Chihuahua: *Nelson* (no. 6054), 1899; *Townsend* and *Barber* (no. 60), 1899, *Goldman* (no. 430), 1899; *Palmer* (no. 309), 1885; *Pringle* (no. 1209), 1887.

Durango: *Palmer* (no. 238), 1896; *Nelson* (no. 4768), 1898.

San Luis Potosi: *Parry* and *Palmer* (no. 135), 1878; *Schaffner* (no. 602), 1879.

Valley of Mexico: *Bourgeau* (no. 79), 1865–66; Pedregal, near Tlalpam, *Rose* and *Hough* (no. 4518), 1899.

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BRIEFER ARTICLES

THE BASIDIUM OF AMANITA BISPORIGERA.[1]

(WITH SEVENTEEN FIGURES)

THERE are among the Hymenomycetes certain species which have basidia bearing only two spores instead of the usual four. Such a form is sometimes found in a genus the other members of which have basidia with the usual number of spores. Some time ago while studying the structure of a white Amanita which resembles *A. verna*, Professor ATKINSON discovered that certain of the plants had basidia with only two spores. He also found that the two-spored plant could be distinguished from the four-spored *A. verna* by other characters, and he has described it as a new species, *A. bisporigera*.

AMANITA BISPORIGERA.

Amanita bisporigera Atkinson, n. sp.—Plants entirely white, usually occurring singly, about 9–13^{cm} high; pileus 4–6.5^{cm} broad; stem 5–8^{mm} thick; bulb 2–2.5^{cm} thick. Pileus convex to expanded, thin, often gibbous or somewhat broadly umbonate, smooth, viscid when moist, thin. Gills subelliptical, usually tapering more behind where they are rounded and free but close, rather crowded, edge of gills floccose. Basidia 2-spored. Spores globose to subglobose or oboval, smooth, with a minute pedicel where attached to the sterigmata as in many species, 8–10 μ. Stem nearly even, or slightly tapering upward, solid, when fresh finely floccose scaly both above and below the annulus, in age tending to become smooth especially below the annulus. Annulus thin, membranous, fragile, sometimes entire, sometimes torn, superior. Volva thick, with apical dehiscence and with the free limb splitting into two or three lobes which are usually closely appressed against the stem.

On the ground in woods. It has been found many times at Ithaca, N. Y. and vicinity, and specimens under twelve or more collections are in the Herbarium of the Department of Botany, Cornell University.

The plant bears a striking resemblance to *A. verna*, but is distinguished by its more slender habit and the two-spored basidia.

As this plant so closely resembles a four-spored species, it seemed desirable to study the nuclear phenomena in the basidium to determine how the behavior of the nuclei compares with what has been observed in the four-spored forms by WAGER (**5**, **6**), JUEL (**4**), HARPER (**2**), and others. The results of a number of investigators make it seem very probable that in all Basidiomycetes the young basidium contains two small primary nuclei which fuse to form the secondary nucleus of the basidium.

[1] Contributions from the Department of Botany, Cornell University, No. 108.

WAGER, however, was of the opinion that in some cases more than two nuclei move into the young basidium from the multinucleate cells of the hyphae. After the fusion of the primary nuclei, the secondary nucleus increases greatly in size. By two successive divisions of this nucleus, four nuclei are produced for the spores. In basidia with more than four spores, as in some Gastromycetes, there are probably more than two divisions. In Dacryomyces, which has basidia with only two spores, there has been some difference of opinion, but it seems probable that the two nuclear divisions take place in the usual way. DANGEARD (1) observed only one division in *D. deliquescens* Bull. ISTVÁNFFI (3) found that in *D. chrysocomus* Bull. there are two successive divisions, and the four nuclei arrange themselves in a row and move in pairs into the branches of the basidium.

However, the young spore receives but one nucleus, as one remains behind in each branch of the basidium. JUEL (4) worked with *D. deliquescens* Bull. and found that there are two successive divisions of the nucleus, but each spore receives a single nucleus, the others remaining behind in the basidium.

The material for this study was secured during the summer of 1904 from plants collected in the vicinity of Ithaca, N. Y. Small pieces were cut from the pilei of young plants soon after their collection and were placed in 1 per cent. chromacetic acid where they remained 12–24 hours. The material was then washed 3–4 hours in running water, dehydrated in grades of alcohol, and passed gradually into paraffin. Sections were cut 3–5 μ in thickness. In sections showing some mature spores several stages in the development of the basidium are found. It is better, however, for the study of the young stages to take sections from younger plants. In fixing material from plants so young that none of the basidia bear sterigmata, there is a possibility of confusing these plants with *A. verna*. To avoid this, small pieces were cut from the pilei of the young plants and these plants were then placed in a moist chamber and allowed to continue their growth until the spores were produced. Then by freehand sections of the pileus the species could be exactly determined. *A. bisporigera* is so distinct in appearance, however, on account of its more slender form, that after one becomes familiar with it there is no difficulty in distinguishing it from *A. verna*, even before it is fully mature.

The sections were stained with safranin and gentian violet, which gives very good results. The preparations were studied with Zeiss 2^{mm} apochromatic, 1.40 aperture, and oculars 8, 12, and 18. Drawings were made with camera lucida and ocular 18.

Material of the two-spored *Agaricus campestris* was prepared for study in the same way, but on account of the smaller size of the nuclei and the dense contents of the basidium, this plant does not offer favorable material.

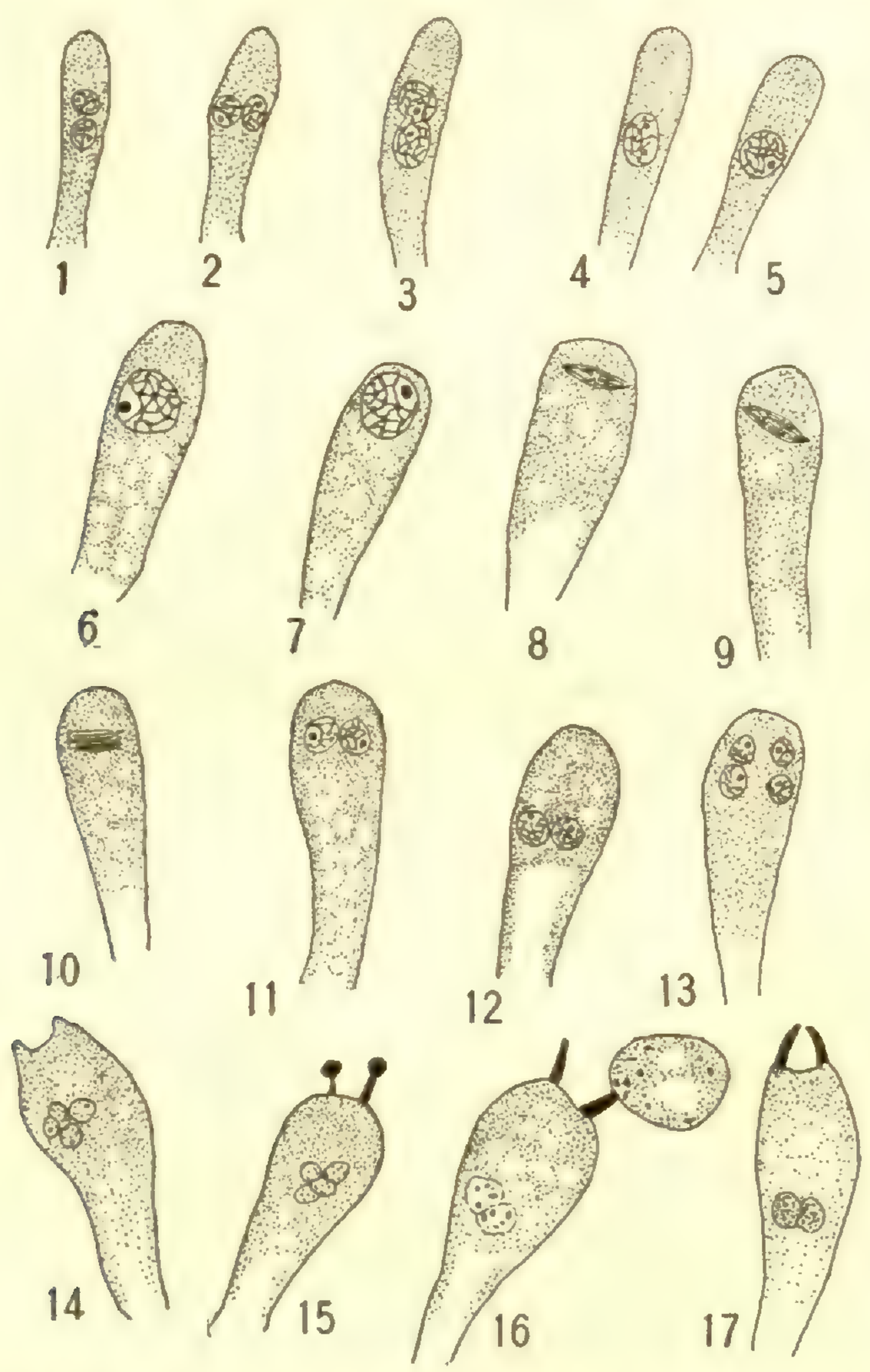

FIG. 1. Young basidium which is densely filled with cytoplasm and contains two primary nuclei.—FIG. 2. The membranes of the two nuclei are in contact.—FIG. 3. Two primary nuclei in the process of fusion, but the nucleoli indistinct.—FIG. 4. Fusion of the nuclei almost complete.—FIG. 5. Basidium somewhat increased in size after fusion of primary nuclei.—FIGS. 6 and 7. Older basidia in which the secondary nucleus occupies a position near the end of the basidium; structure of nucleus at this stage very distinct.—FIG. 8. Division of secondary nucleus.—FIG. 9. Chromosomes moving to the poles of the spindle.—FIG. 10. Chromosomes at poles of the spindle.—FIGS. 11 and 12. Daughter nuclei occupying different positions in basidium; usually near the end.—FIG. 13. Basidium showing four nuclei.—FIG. 14. The four nuclei crowded together at some distance from the end of the basidium and the sterigmata beginning to form.—FIG. 15. The cytoplasm beginning to pass through the sterigmata to form the spores.—FIG. 16. A basidium in which the spores are almost mature; two nuclei still seen near the center of the basidium.—FIG. 17. Old basidium from which spores have fallen; two nuclei near the center.

The young basidium first appears as a club-shaped branch from the sub-hymenial layer. It is more densely filled with protoplasm than in older stages and contains two nuclei. The structure of these nuclei can be made out very easily. Each nucleus has a rather large deeply staining nucleolus, a network in which the chromatin granules are imbedded, and a distinct nuclear membrane.

As the basidium increases in size the nuclei fuse together into one large nucleus. At first the two nuclei lie side by side with their membranes in contact and without apparent change. Then the membranes disappear at the point of contact. The nucleoli remain distinct for a short time, but finally fuse so that the large secondary nucleus produced by the fusion contains but a single nucleolus. The manner of the fusion of the threads bearing the chromatin could not be made out. After this fusion, the nucleus increases in size and comes to occupy a position near the upper expanded end of the basidium (*figs. 6* and *7*).

At this stage the structure of the nucleus can best be determined. It is so large that it fills more than two-thirds the diameter of the basidium. The nuclear membrane is very distinct, so that the nucleus stands out very clearly from the contents of the basidium, which at this stage have become vacuolate. The nucleolus is large and stains deeply, taking a reddish color with the triple stain. The nuclear network consists of one or more coiled threads in which are imbedded the chromatin granules, which stain blue or purple. Between the coils of the thread are the colorless spaces which are filled by the nuclear sap in the living cell.

The nuclear division takes place in the manner described by WAGER (5) for *A. muscaria*. The chromosomes are produced from the thread bearing the chromatin granules. The number of chromosomes is small, but I have been unable to determine the exact number. The chromosomes are small and stain deeply. The spindle consists of a small number of fibers, but they do not show the structures of fibers very clearly because they are crowded closely together. The spindle is arranged transversely near the apex of the basidium and is long and narrow with a small deeply staining body at each pole. Such spindles as the one shown in *fig. 8* are found frequently in the preparations. After the chromosomes move to the poles there are a few persisting spindle fibers which connect them. The daughter nuclei are now formed and usually occupy a position near the apex of the basidium (*fig. 11*). Each daughter nucleus has the same form and structure as the parent nucleus.

The most important question now is whether these two nuclei divide again to produce four nuclei, as is the case in plants with four spores on a

basidium. After careful search I have been unable to find the spindles of such a division, but numerous basidia which contained four nuclei have been observed. In some cases only three nuclei show in the section, but it seems probable that in all such cases the fourth nucleus is in another section. These nuclei are small and in most cases the structure is not so distinct as in the earlier nuclei of the basidium (*fig. 13*). The four nuclei move back from the apex of the basidium and become crowded together in an irregular mass in which it is difficult to distinguish the individual nuclei (*figs. 14* and *15*).

Soon after the four nuclei are formed, the two sterigmata grow out from the end of the basidium. The granular content of the basidium moves up and becomes more dense near the apex. Then the protoplasm begins to pass out through the sterigmata to form the spores. The question which now presents itself is as to the number of nuclei which pass into the spores. It is difficult to follow the details of the passage of the nucleus through the sterigma, and it seems to me that the best evidence as to the number of nuclei which enter the spores is found in the examination of old basidia in which the spores are fully mature or may have fallen off. Such basidia which contain only a small amount of cytoplasm show the presence of two nuclei (*fig. 17*). From the facts that the basidium when the sterigmata are formed contains four nuclei and that it contains only two when the spores are mature one may conclude that two nuclear divisions take place as in those forms with basidia which bear four spores, but that only two of the nuclei enter the spores.—CHARLES E. LEWIS, *Cornell University*.

LITERATURE CITED.

1. DANGEARD, Mémoire sur la réproduction sexuelle des Basidiomycétes. Le Botaniste IV. 1895.
2. HARPER, R. A., Binucleate cells in certain Hymenomycetes. BOT. GAZETTE **33**:1–24. 1902.
3. ISTVÁNFFI, G., Ueber die Rolle der Zellkerne bei der Entwickelung der Pilze. Ber. Deutsch. Bot. Gesell. **13**:——. 1895.
4. JUEL, H. O., Die Kerntheilungen in den Basidien und die Phylogenie der Basidiomyceten. Jahrb. Wiss. Bot. **32**: 361. 1898.
5. WAGER, H., On nuclear division in the Hymenomycetes. Annals of Botany **7**:489. 1893.
6. ———, On the presence of centrospheres in fungi. Ibid. **8**:321. 1894.

CURRENT LITERATURE.

MINOR NOTICES.

The algae.—The second volume of OLTMANNS' large work on the algae has appeared.[1] This part treats of a variety of general topics, the first volume having been devoted especially to the different groups. Among the subjects are the algal cell, the development of reproductive organs, the nourishment of algae, life conditions, response to stimuli, polymorphism, life histories, adaptations, and a discussion of methods of collection, study, and culture. The two volumes give an excellent digest of the large literature in phycology and will prove very valuable as the starting point for many lines of further advance. Some of OLTMANNS' views, as for example that of the place of the tetraspore in the life history of red algae, are not likely to be sustained, but the work is a very scholarly contribution to botanical science and will be welcomed as the only publication of its class in the field of phycology.—B. M. DAVIS.

North American Flora.[2]—The general character and scope of this great work were stated in this journal in connection with the appearance of the first part.[3] Another part has now appeared, being a direct continuation of the former one, and both belong to volume 22 in the general scheme.

Saxifragaceae.—Conimitella, *Elmera*, and *Ocrearia* are established as new genera, and 30 other genera are recognized; new species are described under Lithophragma (8), Tellima, Mitella (2), Pectiantia, Ozomelis, Heuchera (25), Sullivantia, Therophon (3), Saxifraga, Muscaria (4), Micranthes (12), Spatularia, Leptasea (3), and Heterisia.

Hydrangeaceae.—Neodeutzia is established as a new genus, and 9 other genera are recognized; new species are described under Philadelphus (11) and Edwinia (2).—J. M. C.

Philippine plants.—Recent bulletins (nos. 29 and 35) from the Bureau of Government Laboratories show commendable activity in the study of Philippine plants. ELMER D. MERRILL, botanist of the laboratory, is publishing a series of papers on new or noteworthy plants, the third and fourth papers appearing

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[1] OLTMANNS, F., Morphologie und Biologie der Algen. Zweiter Band. Allgemeiner Teil. 8vo. pp. vi+443. Jena: Gustav Fischer. 1905. *M* 12.

[2] North American Flora. Vol. 22. Part 2. Saxifragaceae, Hydrangeaceae, JOHN KUNKEL SMALL, PER AXEL RYDBERG. Cunoniaceae, Iteaceae, Hamamelidaceae, NATHANIEL LORD BRITTON. Petrostemonaceae, PERCY WILSON. Phyllonomaceae, HENRY HURD RUSBY. 8vo. pp. 81–191. New York: The New York Botanical Garden. December 18, 1905. Subscription price $1.50 for each part.

[3] BOT. GAZETTE **40**:74. 1905.

in the bulletins before us, and containing descriptions of nearly 150 new species. There are also notes on the Gramineae by E. HACKEL, including descriptions of 2 new species; an account of the Scitamineae by HENRY N. RIDLEY, 8 new species being characterized; and 10 new species of Acanthaceae by C. B. CLARKE.—J. M. C.

Aster.—In 1902[4] E. S. BURGESS published a "History of Pre-Clusian Botany in its relation to Aster;" and now a second paper on Aster has appeared,[5] which deals with the "Biotian Asters." Under the head of variation, specific limits in the genus are discussed; also normal characters and the comparative variability of organs. There is no group of flowering plants in which such a discussion would seem more difficult. A systematic treatment of the species is also begun, 84 species being presented with great fullness, 58 of which are published for the first time; also 10 subspecies and about 250 subordinate forms are characterized.—J. M. C.

Festuca.—C. V. PIPER[6] has published a monograph of the North American species of Festuca, recognizing 34 species, and characterizing 3 of them as new. A third subgenus is added to Vulpia and Eufestuca, to include *F. confinis* Vasey, and is called *Hesperochloa*. There are also notes on several Mexican species including descriptions of 2 new species. A new word is added to the terminology of grasses. The word "glume" is restricted to the "empty glumes;" while the "lower palet" or "outer palet" or "flowering glume" of authors is the *lemma*, a Greek word meaning husk or scale.—J. M. C.

Plants of the Bahamas.—Dr. C. F. MILLSPAUGH, Field Columbian Museum, has issued the first paper[7] of a series dealing with the flora of the Bahamas, Amaranthaceae, Euphorbiaceae, Rubiaceae, and Verbenaceae are presented, and a new species of Solanum is described. New species are also described under Iresine (2), Argythamnia (2), Euphorbia (3), Chiococca, Lantana, Valerianodes, and Callicarpa; and two new genera (*Nashia* and *Pseudocarpidium*) of Verbenaceae are established.—J. M. C.

Lichens of Santa Cruz.—A. W. C. R. HERRE[8] has published an account of the foliaceous and fruticose lichens of the Santa Cruz peninsula, which is a natural biological region lying west of San Francisco Bay and extending south-

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[4] Mem. Torr. Bot. Club, 10.

[5] BURGESS, EDWARD SANFORD, Species and variations of Biotian Asters, with discussion of variability in Aster. Mem. Torr. Bot. Club **13**: 419. *figs.* 108. 1906.

[6] PIPER, CHARLES V., North American species of Festuca. Contrib. U. S. Nat. Herb. **10**: 1–48. *pls. 1–15.* 1906.

[7] MILLSPAUGH, C. F., Praenunciae Bahamenses. I. Field Columb. Mus. Bot. **2**: 137–184. 1906.

[8] HERRE, ALBERT W. C. T., The foliaceous and fruticose lichens of the Santa Cruz peninsula, California. Proc. Wash. Acad. Sci. **7**: 325–396. 1906.

ward to Monterey Bay. Species are described under 22 genera, Parmelia being the largest with 14 species; and new species are characterized under Cetraria, Usnea, Parmelia, and Gyrophra.—J. M. C.

Die natürlichen Pflanzenfamilien.—Part 223 continues the families of mosses by BROTHERUS, Hedwigiaceae being concluded; Fontinalaceae, Climaciaceae, Cryphaeaceae, Leucodontaceae, and Prionodontaceae being completed; and Spiridentaceae being begun.

The first part of the second supplement has also appeared, including the literature of 1899–1904 in reference to gymnosperms and monocotyledons, with a few pages beginning the dicotyledons.—J. M. C.

Index Filicum.—The sixth, seventh, and eighth fascicles of CHRISTENSEN'S work[9] have appeared with great promptness, carrying the references from *Gleichenia Cunninghamii* to *Polypodium basiattenuatum*. It should be urged upon colleges and libraries that so useful and thankless a task should be supported by adequate subscriptions.—J. M. C.

Text-book of pharmacognosy.—A new textbook of pharmacognosy by GILG[10] is worthy an English edition. It is the best illustrated text for ordinary student use that has appeared. The work would be still more valuable if a greater number of cuts showing the anatomical elements as they appear in powder had been included.—RAYMOND H. POND.

Plants of Bermuda.—A list of plants collected by the author in Bermuda in 1905 has been published privately by A. H. MOORE of Cambridge, Mass. The pamphlet contains 22 pages, 3 plate reproductions of photographs, and descriptions of new species of Rhynchospora and Elaeodendron.—J. M. C.

Das Pflanzenreich.[11]—Part 24, issued in January of this year, contains the Aponogetonaceae by KRAUSE and ENGLER, 22 species being recognized.—J. M. C.

NOTES FOR STUDENTS.

Items of taxonomic interest.—J. CARDOT continues (Bull. Herb. Boiss. II. **6**: 1–17. 1906) his account of the mosses collected by the Swedish Antarctic Expedition, describing nineteen new species from S. Georgia Island and 5 from the Antarctic lands.—PALIBRIN adds (*idem* 18–22) 5 new species to the Chinese flora.—H. CHRIST lists (*idem* 45–58) the ferns of Costa Rica, which is astonishingly rich, and describes 8 as new.—I. THÉRIOT (Bull. Acad. Int. Geog. Bot. **16**: 40. 1906) gives a 2-line diagnosis of two new Leptodontia from New Granada, with other

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[9] CHRISTENSEN, C., Index Filicum, etc. Fasc. 6–8. Copenhagen: H. Hagerups Boghandel. 1905 and 1906. Each 3*s*. 6*d*.

[10] GILG, ERNEST, Lehrbuch der Pharmacognosie. 8vo, pp. vii + 368. Berlin: Julius Springer. 1905.

[11] ENGLER, A., Das Pflanzenreich. Heft. 24, Aponogetonaceae by K. KRAUSE assisted by A. ENGLER. pp. 22, *figs.* 9 (71). *M* 1.20. Leipzig: Wilhelm Engelmann. 1906.

new species from China.—E. L. GREENE characterizes (Ottawa Nat. **19**:197. 1906) a new Antennaria from Athabasca.—F. VON HÖHNEL describes (Ann. Mycologici **3**:404. 1905) a new genus, *Unguicularia*, near Pezizella, and 3 new species of fungi.—E. BRAINERD adds 2 new names to New England violets (Rhodora **7**:245–7. 1905).—M. L. FERNALD (*idem* 250) differentiates from *Cynoglossum virginicum* a new species, *C. boreale*.—J. A. CUSHMAN concludes (*idem* 251–266) his enumeration of the desmids of New Hampshire, listing 253 species and varieties against the 74 hitherto reported. He describes several new ones.—M. L. FERNALD (*idem* **8**:11 and 22. 1906) describes a new Geum from Vermont and a new Salix from Maine; attempts to clear up (*idem* 31) the American forms called *Arenaria verna*, including a new species; and characterizes (*idem* 69–71) 2 new species of Streptopus.—In a presentation of Astragalus and its segregates as represented in Colorado, P. A. RYDBERG (Bull. Torr. Bot. Club **32**:657–668. 1905) recognizes 17 genera, 7 of which (*Atelophragma*, *Jonesiella*, *Phacopsis*, *Ctenophyllum*, *Microphacos*, *Cnemidophacos*, and *Diholcos*) are characterized as new; and in his 16th paper on the Rocky Mt. flora (*idem* **33**:137–161. 1906) he describes new species under Rumex, Sphaeralcea, and Senecio (6); establishes as new genera *Crunocallis*, *Naiocrene*, *Erocallis* (all three near Claytonia), *Cornella* (Cornaceae), *Oreochrysum*, *Platyschkuhria*, *Chamaechaenactis*, and *Prenanthella* (all four Compositae).—In his 6th paper on the Hepaticae of Puerto Rico (*idem* 1–25), A. W. EVANS establishes *Rectolejeunea* and *Cystolejeunea* as new genera.—In a 5th paper describing new species of Uredineae (*idem* 27–34) J. C. ARTHUR establishes the new genus *Ceratelium*.—J. K. SMALL (*idem* 51–57), in a 2d paper on N. Am. Polygonaceae, describes new species under Eriogonum (8) and Polygonum.—L. M. UNDERWOOD and F. E. LLOYD (*idem* 101–124) describe 17 new species of Lycopodium from the American tropics.—L. M. UNDERWOOD (*idem* 189–205) characterizes new species of pteridophytes from the United States under Asplenium (2), Stenochlaena, Tectaria, and Selaginella.—R. SCHLECHTER (Engler's Bot. Jahrb. **39**:1–100. 1906) in a study of the flora of New Caledonia establishes 3 new genera (*Coilochilus*, *Pachyplectron*, *Gonatostylis*) of Orchidaceae and one (*Trilocularia*) of Balanopsidaceae.—U. DAMMER (*idem* 20–22) describes *Actinokentia* and *Nephrocarpus* as new genera of New Caledonian palms.—W. SUKSDORF (Oesterr. Bot. Zeits. **12**:5–7, 26, 27. 1906) has described new species of Washington plants under Sanicula, Lomatium, Antennaria, Lasthenia, Pyrola, Navaretia, Orthocarpus, and Aphyllon (2).—A. A. HELLER (Muhlenbergia **2**:1–164. 1905–6) has published an account of his Californian collections during 1905, including descriptions of new species under Eriogonum (3), Montia, Delphinium, Ranunculus, Thysanocarpus (2), Lithophragma, Ribes, Amelanchier, Lupinus (14), Vicia, Acrolasia, Boisduvalia, Glaux, Apocynum, Gilia (4), Solanum, Pentstemon, Castilleia, Orthocarpus, and Scorzonella; and of 2 new genera, *Hesperastragalus* (Leguminosae) and *Malacolepis* (Compositae).—MAX FLEISCHER (Hedwigia **45**:65–87. 1906), in concluding his paper on new families, genera, and species of mosses, describes *Baldwiniella*, *Homaliodendron*, *Pinnatella*, and *Penzigiella* as new genera of

Neckeraceae.—U. DAMMER (Notizblatt König. Bot. Gart. **4**: 171–173. 1905) describes a new genus (*Kinetostigma*) of Guatemalan palms.—E. JANCZEWSKI (Bull. Acad. Sci. Cracovie, pp. 13. Jan. 1906), in his second paper on Ribes, presents the species of the subgenera Ribesia and Coreosma, including new Californian and Mexican species.—A. BORZI (Notarisia **21**: 14–16. 1906) describes a new genus (*Zoddaea*) of Chlorophyceae (Chroolepidaceae) from a Mediterranean island.

Heredity.—A lecture on heredity and the origin of species by MACDOUGAL[12] not only presents the author's views regarding the several more prominent evolution hypotheses, but also makes the first public announcement of important results of his own researches on the causes of mutation.

While not denying the possibility of other means of production of species, he holds that hybridization and mutation are the only *demonstrated* methods by which new species have arisen. He attributes a popular belief in the Neo-Lamarckian hypothesis to the supposed effects of garden practice, and these supposed effects are supposed to be due to the prevalence of vicinism and the vegetative propagation of bud-sports. Several "unsurmountable objections" are opposed to the Neo-Darwinian hypothesis of natural selection of slight variations as a universal method. He would distinguish orthogenesis from determinate variations, limiting the former to an internal perfecting force which evolves rudimentary organs and develops them to functional structures without any reference to selection; while the latter he would allow as a part of every method of evolutionary procedure, in that no structure may vary to any other structure too much unlike itself. This is a very important discrimination theoretically, but it is clear that in most cases a practical distinction between orthogenesis and determinate variation as here defined would be an impossibility, since the "morphological possibilities" may be estimated only by what does appear.

The effects of isolation and of self- and cross-fertilization are held to be problematical.

The greater part of the lecture is naturally devoted to the mutation cultures of DE VRIES and himself. Besides *Oenothera Lamarckiana*, the following three species have been shown to be in a state of mutation: *O. grandiflora*, *O. biennis*, and *O. cruciata*. "Parallel mutations" are exemplified by two observed origins of *nanella*-forms, i. e., forms with linear petals. A consideration of the mutating and mutant species leads to the conclusion that plants are made up of complex groups of unit characters, that some of these characters may exist for an indefinite time in a latent state, that a new character that departs widely from the parental condition is more variable than the homologous character of the parent species, and that at the same time it is less closely correlated.

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[12] MACDOUGAL, D. T., Heredity and the origin of species. Lecture given before the Barnard Botanical Club, Columbia University, December 18, 1905. The Monist, Jan. 1906. 32 pages. Printed and distributed in advance.

The author substitutes for a *period* of mutation the conception of a nearly constant *frequency* of mutation. Thus, one plant in twenty of *O. Lamarckiana* is a mutant, but only one in two hundred of *O. biennis*. In others there may be one in ten thousand or one in a million.

Doubtless the most important fact presented is the result of investigations to determine the cause or causes of mutation. The introduction of strong osmotic and weak chemical solutions into the ovaries of *Raimannia odorata* shortly before fertilization, appears to have produced a large number of individuals of a hitherto unknown type. These new plants have a shorter life-cycle than that of the parent and are profoundly different in many characters. They have already bloomed and fruited, and obviously constitute a potential species. If this new species holds its characters in succeeding generations, this discovery will be one of far-reaching importance, as the first real clue to the causes which may effect mutative changes in plants.—GEORGE H. SHULL.

Graft-hybrids.—NOLL has made a careful morphological, anatomical, and cytological examination of the supposed graft-hybrids between *Crataegus monogyna* (stock) and *Mespilus germanica* (scion) in the Dardar Garden at Bronvaux near Metz, Germany.[13] Three branches, starting from the callus where stock and scion meet, present unmistakable evidence of their hybrid origin, each branch showing a different combination of the parental characters.

A consideration as to the possibility of graft-hybrids, in the light of present knowledge of the behavior of the hereditary substance, leads to the conclusion that they must originate through nuclear fusions in the callus or not at all; and moreover, that the studies of NĚMEC upon asexual nuclear fusions gives a basis of observed fact which warrants the affirmation that graft-hybrids are possible.

The cytological examination of the several hybrid branches showed that their cells do not possess double the normal sporophyte number of chromosomes; therefore, if these hybrids originated from the fusion of two vegetative cells, this process must have been followed by some method of chromosome reduction. This presents no insurmountable difficulty, since NĚMEC found that after 78 hours no nuclei were found which had more than the normal number of chromosomes, though many such were observed soon after fusion.

The greater resemblance of one of the hybrids to Mespilus, and of the other two to Crataegus, and the change of one of the latter from nearly typical Crataegus to near one of the other hybrid forms, are explained by assuming that in each fusion one nucleus remained in its accustomed cytoplasmic surroundings, and that the other nucleus, moving into unaccustomed surroundings, was so weakened or injured that, when the degeneration took place which reduced the chromosomes to their normal number, the weakened or injured chromosomes contributed the fewest determinants to the hybrid nucleus, thus giving the hybrid

[13] NOLL, T., Die Pfropf-Bastarde von Bronvaux. Sitzungsber. Niederrhein. Ges. f. Natur-u. Heilkunde Bonn, 1905. Separate, 34 pp.

a greater resemblance to the species furnishing the stationary or "mother-" nucleus.

The investigation shows that neither stock nor scion is itself of hybrid origin and that there can be no reasonable doubt that these are true graft-hybrids.

The only other similar case that has attracted much attention is that of *Laburnum* (*Cytisus*) *Adami*, and about this plant there has been so much contention that, in the absence of other authentic graft-hybrids and with the disappearance of the original tree, it seemed best to many botanists to consider the original observation and record to be in error. NOLL prints the original account in full, and decides, after considering the possible sources of error and misinterpretation, that the internal evidence in favor of this statement compels belief in its truth.

The final demonstration must lie in a reconstruction of the same or a similar hybrid, experimentally, and on this work NOLL has been engaged for a number of years, as yet with wholly negative results; but the rarity of the phenomenon makes this quite to be expected, and the author still hopes by improving his technic to succeed in re-creating *Laburnum Adami*.—GEORGE H. SHULL.

Experimental variation.—KLEBS[14] presents a paper which deserves special attention because of the experimental data recorded, because of the author's effort to make a closer analysis of the problem of experimental variation, and because a substitute for DE VRIES'S intracellular pangenesis is offered. Long experience with the behavior of algae and fungi under artificial conditions, probably as much as the results stated in this paper, has convinced the author that in the last analysis all variations must be referred to the influence which external factors exert upon the inherent potencies of the organism. From this point of view the fundamental problem of experimental variation at once appears to be to determine the potential amplitude of variation for species. This problem is to be solved by the application of as great a variety of conditions as possible. Some of those used by the author are temperature, darkness, wounding, and artificial food. The results obtained with *Campanula trachelium* and *Sempervivum Funkii* show that the accepted taxonomic limits of a given species are easily transgressed whenever external conditions favor the expression of potencies inherent in the organism. Trial clearly shows that the potency of external conditions is much greater before the inception of organs than after. If, for example, nutrition is the determining factor for a given variation, it makes little difference whether the necessary nutrition status is established by one external condition or another.

Over one hundred pages are used to expound the author's view of the correlation of variation and environment and to present a polemic criticism of intracellular pangenesis. The results with *Sempervivum Funkii* show that those characters which can appear as specific within the genus can by proper method be induced to appear upon a single species. A species therefore is to be

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[14] KLEBS, GEORG, Ueber Variationen der Blüten. Jahrb. Wiss. Bot. **42**: 155–320. *pl. 1. figs. 27.* 1906.

characterized only by its constant relation to the outer world, and the author believes any other definition is artificial and arbitrary. More explicitly, a species is defined as comprising all those individuals which have arisen by vegetative reproduction or by self fertilization, and which for many generations under like conditions have shown identical characters. This definition is not arbitrary to the reviewer, provided the assumption of a specific structure upon which it rests is not arbitrary. If by definition potencies can never transgress the limit prescribed by the specific structure and variation is merely the expression of potencies, how have species arisen by variation? The potencies of the author are merely inherent capacities to respond to certain combinations of external conditions and are purely immaterial as compared with the pangens of DE VRIES, which are material and carry the unit characters. To some investigators this discrimination will probably appeal as being an interpretation closer to nature and more logical from the strictly physiological standpoint. To others it may seem as merely a restatement of the conception of DE VRIES. The latter might easily inquire what difference it makes whether a given variation has arisen by an inactive pangen becoming active or by a hitherto impotent potency becoming potent.—RAYMOND H. POND.

The lakes of Scotland and Denmark.—At the invitation of Sir JOHN MURRAY, DR. C. WESENBERG-LUND[15] spent three or four weeks on the Scottish lakes in order to make a comparison with the lakes of Denmark. While this was a short time in which to make examination of a new country nevertheless we should expect interesting results from one who has not only accomplished so much thoroughly good work in the study of lakes, but has shown unusual skill and originality in his interpretations.

The general differences which the author finds between the Danish and Scottish lakes are the differences which we should expect between shallow and deep lakes. The Danish lakes have more plankton, more floating and submerged vegetation, and more distinct littoral zones of vegetation. The greater seasonal variations in the Danish lakes is noted; this, of course, would be expected from the greater variations in temperature. There are more highly colored crustacea in the Scottish than in the Danish lakes. The reviewer thinks the author right in correlating this red color with low temperature, rather than with elevation as has been done by some other authors.

Among the diatoms the author notes the absence of Melosira and Stephanodiscus in the Scottish lakes, with an abundance of Asterionella and Tabellaria. These facts compare well with the differences in America between the deep and shallow lakes. The Scottish lakes are remarkable for the large number of desmids. These desmids are of forms that are common in the pools of the hillsides. The occurrence of these desmids in the plankton, together

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[15] WESENBERG-LUND, C., A comparative study of the lakes of Scotland and Denmark. Proc. Roy. Soc. Edinburgh **22**:401–488. *pls. 2.* 1905.

with the occurrence of Entomostraca that are also common in pools, leads the author to the generalization that the limnetic plankton of the Scottish lakes is of littoral origin, and that the transportation of these forms to become a part of the limnetic fauna and flora is favored by the steep hillsides surrounding the lakes, and the extremely narrow littoral region.

The author enters upon a somewhat detailed discussion of the influence of the organic life upon the lakes themselves, showing how in the Danish lakes the algae and higher plants make deposits of lime which are partly thrown upon the beach, and partly fall to the bottom in the limnetic region. In these bottom deposits it is again worked over by worms and insect larvae, which devour the remaining organic matter and leave the bottom sometimes composed almost entirely of lime and clay. In the Scottish lakes the bottom in the deeper portions is composed of material largely derived from the littoral and shore regions, and there is an absence of lime.

The general conclusion is that while the Danish lakes are filling up, the Scottish lakes will remain with very slight alteration for ages.—C. DWIGHT MARSH.

Chlorosis.—One of the most notable papers recently published on the type of diseases which may be classed as chlorosis is that of BAUR on the infectious chlorosis of the Malvaceae. The variegated mallows in cultivation were derived from a form of *Abutilon striatum* known as *A. Thomsoni*, which appeared in a collection of A. *striatum* imported into England from the West Indies in 1868. This plant was found to be capable of transmitting its variegation by grafting. BAUR finds that if the leaves are removed from variegated plants, or if the shoots are cut back so that no leaves remain and the plants kept in the dark, new shoots form only two or three variegated leaves, and if those are removed the plants remain permanently green in the light unless they are again infected from scions of variegated plants. However, if latent axillary buds on the old parts are forced into growth, these produce shoots with variegated leaves which in turn infect all newly formed leaves on the plant. When all variegated leaves are removed from a plant exposed to light, the plant becomes permanently green. Similarly when scions of the green but susceptible *A. arboreum* are grafted on defoliated variegated plants, the scions remain green, but here also if a variegated shoot is allowed to develop from the stock it rapidly infects the whole plant. The author concludes that the variegation in these plants is caused by a substance or virus which is formed only in the light in the chlorotic parts of the plants; that this virus is produced only in small excess so that it is rapidly used up if the variegated leaves are continually removed. The substance is capable of infecting only the embryonic leaves and in those it is stored for months in an inactive form. By appropriate girdling and grafting experiments the approximate rate of movement and the path followed was determined. Movement takes place in the cortex and not with the transpiration stream. When scions of immune *A. arboreum* are grafted on a variegated *A. Thomsoni*, they grow vigorously but are not infected;

but if scions of some susceptible species are grafted on the former these become infected, showing that the virus can pass unchanged through the intermediate piece of *A. arboreum*. These experiments seem to prove the existence in the plant of a substance which in its behavior is analogous to the supposed shoot-forming substance of SACHS, or the growth enzymes of BEYERINCK.—H. HASSELBRING.

Anatomy as a test of species.—ALFRED SARTON[16] has made an elaborate experimental study of the anatomy of related plants, to test the constancy of anatomical characters under varying conditions of climate and of soil. The work was done at the Botanical Laboratory of the Sorbonne and at the Laboratory of Plant Biology at Fontainebleau. He calls attention to the fact that there are two kinds of species recognized in taxonomic writings: one he calls the "Linnaean species," which often bring together under a single name a large number of different forms; the other he calls the "Jordanian species," which often consist of dismembered Linnaean species. These two kinds stand side by side as of equal rank, all of them based upon varying judgments as to the value of external morphological characters.

SARTON set out to discover whether real species could be detected by their anatomical characters. He reasons that nearly allied species whose anatomical differences may be exchanged under experiment are not separate species, however unlike they may appear externally; and that those whose anatomical differences are constant under experiment are true species, however similar they may appear externally. To test this dictum involved a large amount of laborious experimentation and anatomical investigation. The result was to pronounce some Jordanian species good and others not; and the Linnaean species shared the same fate. This anatomical method, therefore, furnishes no basis for judgment between the two types of species; and if it is used, it seems to the reviewer that it will result in readjusting specific lines without settling anything.

The fundamental weakness in this whole point of view is the idea that there can be any rigid test for that elusive conception known as a "species" which will carry it beyond the reach of fallible and hence diverse human judgment. It is of great interest to know what anatomical characters will vary under given conditions, and herein lies the chief value of this investigation; but even here the conditions are not analyzed so as to be convincing. To regard these characters as outweighing all others is to stir afresh the seething mess of taxonomy. What we need is not more "specifics" but more hygiene.—J. M. C.

Transpiration of evergreens.—PUGLISI[17] has published a paper on the transpiration of seven species of Chinese and Japanese evergreen trees and shrubs.

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[16] SARTON, ALFRED, Recherches expérimentales sur l'anatomie des plantes affines. Ann. Sci. Nat. Bot. IX. **2**:1–115. *pls. 1–4.* 1905.

[17] PUGLISI, M., Sulla transpirazione di alcune piante a foglie sempreverdi. Annali di Botanica **2**:435–468. *pl. 2.* 1905.

His objects were (1) to compare the winter transpiration of these species in Rome with that already observed by KUSANO in Tokio during the winter; and (2) to compare the winter and summer transpiration in Rome. One set of experiments was made with single leaves exposed 24 hours in GARREAU'S apparatus, and another series with small twigs (in one case a leaf only) attached to MOLL potometers. The potometer measurements were usually continued for about 8 days and readings made at 9 A. M. and 5 P. M.

The author found the winter rate of transpiration at Rome decidedly greater than that at Tokio. He obtained for an average value of the ratio of winter to summer transpiration at Rome, for all the species examined, 1:3.10. The average ratio at Tokio of KUSANO'S "typical plants" (species not given) was 1:20.

PUGLISI reports that transpiration continued at night during the minimum temperature of his experiments, 2.6°. The rate of transpiration continued to increase with the rise of temperature up to and including the hottest days of July in which experiments were made.

There is a notable difference among the plants experimented upon in the sensitiveness to change of temperature. Measured by GARREAU'S method, *Ficus erecta* showed an increase of 1.5 times in the amount of transpiration when the temperature rose from 6.4° to 21.6°, and *Raphiolepis japonica* showed an increase of 7 times for about a degree more of rise than that of the Ficus.

The paper contains many interesting data, but the author has not sufficiently summarized his results. It seems unfortunate that so much stress is laid on observations made under the highly unnatural conditions imposed by the GARREAU method. All of the winter measurements were made by this method, which eliminates the effect of changes in relative humidity at a season when the actual range of this factor was from 58 to 95 per cent.—J. Y. BERGEN.

Plant breeding in the tropics.—LOCK[18] gives a further account of his studies in plant breeding at Peradeniya, Ceylon. His general conclusions were given in an earlier paper, and the present contribution describes in detail the experiments with the genus Pisum. Records of climatic conditions are given, and the changes which were induced in various European varieties on introduction into Ceylon. There was no gradual adjustment or acclimatization, the change of stature, habit, etc., being immediate and permanent during the several years of the investigations.

In all of the experiments wherein the characters are clearly alternate, the agreement with theoretical ratios is as close as the numbers used would warrant one to expect, on the hypothesis that the union of gametes bearing the several characters is purely a matter of chance. The author greatly weakens his paper, however, by pointing out Mendelian ratios where they are wholly unwarranted by his data, as for instance in width of pod (p. 371), where a variation curve with

[18] LOCK, R. H., Studies in plant breeding in the tropics. Ann. Roy. Bot. Gardens Peradeniya 2:357–414. 1905.

millimeter classes showing the frequencies 1, 6, 5, 8, 4, 5, 2, 1, is grouped in a ratio of 7 wide : 17 medium : 8 narrow or nearly the expected 1:2:1. Every variation curve of purely chance variates can be arranged in this way by counting one-fourth of all the variates from each extreme, leaving the group between the quartiles as the 50 per cent. intermediates expected.

The author reaffirms in a general statement the explanation offered in his first paper,[19] to account for the appearance of certain *nova;* but makes an interesting observation in disagreement with that explanation, apparently without noting the discrepancy—the new character of the pigmented parent which was changed to the active state by crossing. He now states that he could occasionally observe the mottled pattern like a faint water mark in the *white* parent, and its occurrence there makes this an excellent new evidence that the mottled character is not latent in the usual sense of being *inactive,* and that it is *not* present in the pigmented parent, but being possessed by the *white* parent is simply *invisible* owing to the lack of pigment.—GEORGE H. SHULL.

Spraying potatoes.—STEWART, EUSTACE, and SIRRINE[20] have published the extensive results secured by them during 1904 in their series of experiments in the prevention of potato diseases by spraying. The results secured during previous years should be read in this connection.[21] During 1904 a total of fifty-eight experiments were conducted; a few of these were upon the grounds of the Experiment Station at Geneva, while the remainder were conducted as "farmers' business experiments" in various parts of the state. The experiments at Geneva form a part of a ten-year series of experiments designed to give average results for various seasons. The other experiments should yield valuable data year after year as to the actual net gains to be expected from the spraying against potato diseases under actual farm conditions. At Geneva five sprayings increased the yield 233 bushels per acre, while a gain of 191 bushels was secured from three sprayings. This gain was mostly due to the longer growth of the plants made possible by the prevention of the late blight and the rot which follows it. In the business experiments, covering a total of 180 acres, the net gain per acre due to spraying was $24.86. The average loss from blight in New York State during 1904 was not less than 60 bushels per acre. The suggestion is made that the community hire some person to do all their spraying, thus effecting a saving of time and labor.—E. MEAD WILCOX.

Alternation of generations in Phaeophyceae.—Strasburger[22] agrees with OLTMANNS that there is no alternation of generations in the Phaeosporeae. He

[19] See BOT. GAZETTE **39**:303–304. 1905.

[20] STEWART, F. C., EUSTACE, H. J., and SIRRINE, F. A., Potato spraying experiments in 1904. Bull. N. Y. Geneva Exp. Stat. 264:93–204. *pls. 1–16. 1 map.* 1905.

[21] Potato spraying experiments in 1902. Bull. N. Y. Geneva Exp. Stat. 221: 235–263. 1902.

Potato spraying experiments in 1903. Bull. N. Y. Geneva Exp. Stat. 241. 1903.

[22] STRASBURGER, EDWARD, Zur Frage eines Generationswechsels bei Phaeophyceen. Bot. Zeit. **64**: 1–7. 1906.

believes that here, and also in the Chlorophyceae, the germination of the zygote will be found to be accompanied by a reduction of chromosomes, and that consequently a diploid, or *2x* generation cannot be present. The absence of a diploid generation explains why parthenogenesis occurs so readily. Forms like the Dictyotaceae, which have a diploid generation, must be widely separated phylogenetically from the Phaeosporeae. The thallus of the Fucaceae is diploid, while from the initials of oogonia and antheridia to the mature eggs and sperms the condition is haploid, or gametophytic. The rather surprising view is expressed that the antheridia and oogonia of the Fucaceae are not homologous with those of the Dictyotaceae, but that they correspond rather to the tetraspore condition of the latter group. STRASBURGER makes the statement that the gametophytic generation begins with the complete separation of the *2x* (*doppelzahligen*) chromosomes, because this separation furnishes the condition for the formation of sexual products. He does not indicate any more definitely that he would regard the spore rather than the spore mother-cell as the first term of the gametophyte.—CHARLES J. CHAMBERLAIN.

Diseases of sugar cane.—LEWTON-BRAIN finds that the root-disease of the sugar cane in Hawaii[23] is probably due to the fungus *Marasmius sacchari*, known to cause a similar disease in other countries. In Hawaii the fruiting body of the fungus has not yet been found. The Yellow Caledonia variety seems to be resistant to the disease while the Lahaina and Rose Bamboo are most severely injured. Ratoons are more injured than plant canes. Since this fungus is a soil-infesting fungus it may be controlled by liming the soil and through cultivation.

COBB[11] has recently published suggestions as to the inspection and disinfection of sugar cane cuttings to prevent the spread of sugar cane diseases.[24] The cuttings should be made with care to prevent the shattering of the ends, which permits the entrance of fungus parasites, and they should then be carefully inspected to get rid of any diseased ones that may be present. "Pickling" the cuttings in Bordeaux mixture is recommended, and a large part of the paper is devoted to methods of doing this work on the large scale required on a sugar plantation. The cuttings may also be sprayed with Bordeaux mixture in the ditch just before being covered.—E. MEAD WILCOX.

Asparagus rust.—SMITH, as a result of his further studies of the asparagus rust problem on the Pacific coast, finds that the rust may be effectively controlled or entirely prevented by the proper application of a dust spray of flowers of sulfur.[25]

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[23] LEWTON-BRAIN, L., Preliminary notes on root disease of sugar cane in Hawaii. Div. Path. and Phys. Exp. Stat. Hawaiian Sugar Planters' Association Bull. 2:1–39. *figs. 12.* 1905.

[24] COBB, N. A., The inspection and disinfection of cane cuttings. Div. Path. and Phys. Exp. Stat. of the Hawaiian Sugar Planters' Association Bull. 1:1–35, *pls. 8.* 1905.

[25] SMITH, R. E., Further experience in asparagus rust control. Calif. Exp. Stat. Bull. 172:1–21. *figs. 7.* 1906.

The important thing is to get the tops of the plants covered with a perfect coating of the sulfur dust before the rust appears; the first application should be made as soon as the tops have made some little growth, say about three weeks after cutting stops, and a second and perhaps a third application should follow at intervals of about one month each. In some cases it may prove advisable to spray the plants first with some soap spray, to be followed by the sulfur dust to insure the sulfur adhering to the plants. If the best grade of flowers of sulfur be employed, it may be possible to cover an acre with about fifty cents worth. SMITH insists also upon the supreme importance of destroying all wild asparagus plants near the regular fields, since on these the rust first appears, and from them the field soon becomes infested.—E. MEAD WILCOX.

Germination in myxomycetes.—In a preliminary paper on the germination of the spores of myxomycetes, JAHN recognizes two distinct types of germination.[26] The first type is represented by a single species of Ceratiomyxa, whose spore contains four nuclei, the spore content escaping as an amoeba which immediately divides into eight swarmspores. In the second type, embracing all other myxomycetes, the spores are uninucleate and produce a single swarmspore. Regarding the conditions of germination, the following observations are made. The amoeba escapes by rupturing the spore-membrane by osmotic pressure, and if this is increased germination is prevented. The spores of Stemonitis do not germinate when placed in water, but if after being soaked they are allowed to dry they will germinate readily in water, an observation formerly made by LISTER. JAHN concludes that such spores contain a latent enzyme which is made active by the process of moistening and drying. Since maltose but not other sugars causes germination, this assumption is strengthened, as maltose is the decomposition product produced from glycogen stored in the spores.—H. HASSELBRING.

Blight canker.—WHETZEL has published the results of his study of a canker of apple trees in New York state[28] due to the same organism, *Bacillus amylovorus*, that is responsible for the well-known fire blight of apples and pears. This canker has been known in a general way for some years, but this seems to be the first demonstration, by the usual inoculation experiments, of the bacterial nature of the disease. Infection seems to take place only through wounds, and these may be due to pruning, to accidental wounding or "barking" of the tree, or to the work of insects. The germ also enters at times through "water sprouts," since cankers are often seen to appear about the base of such blighted shoots. Pear trees known to be affected with the blight should not be allowed to remain in the neighborhood of an apple orchard, and great care chould be taken to prevent the dissemination of the germs on the pruning instruments. Some variation in resistance to the canker was noted.—E. MEAD WILCOX.

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[27] JAHN, E., Myxomycetenstudien. Ber. Deutsch. Bot. Gesells. **23**:489–497. 1906.

[28] WHETZEL, H. H., The blight canker of apple trees. Bull. Cornell Exp. Sta. 236:99–138. *figs. 51–84.* 1906.

Excretion of acids by roots.—KUNZE[29] has extended the work of MOLISCH, PRIANISCHNIKOW, and CZAPEK on the general chemistry of the excretion of acids by roots, including a study of similar activity as exhibited by mycorhizal fungi. CZAPEK's conclusion that the excretions do not contain free mineral acids is confirmed, but the presence of acid salts of the mineral acids is denied and the corrosive effect is attributed exclusively to the organic acids. Tests with about two hundred different species widely separated in systematic standing shows that many plants do not excrete enough acid from the roots to be detected by litmus. Fungi excrete much more actively and it seems probable that they are more potent as soil disintegrators than the roots of higher plants. The author's work tends to increase his belief in mycorhizal symbiosis. Whether intentional or not it seems a serious deficiency to omit the date from 23 of the total number of 35 citations.—RAYMOND H. POND.

Algae of northern seas.—An interesting paper by SIMMONS[30] discusses the history and relation of the algal floras of the North Atlantic and North Pacific to one another and to that of the Polar Sea. The Atlantic and Pacific Oceans are believed to have received a large number of species from the Polar Sea of Tertiary times, especially just before the ice age, when the algae were driven southward. Some of these became established and never returned to their old situations, but settled and became variously modified in their new life conditions. Others re-entered the Polar Sea with the retreat of the ice. This is a paper which will bear careful study by those familiar with the algal floras of the North Atlantic and Pacific, especially in comparison with BÖRGESEN's *Algal vegetation of the Faeröese coasts* noticed in this journal **41**:71. 1906.—B. M. DAVIS.

Grape diseases.—BUTLER[31] has published some observations on three grape diseases: red-leaf, shrivel, and root-rot. The red-leaf seems to be a disease closely related to diseases known as *folletage*, *rougeot*, and California disease. Like the other diseases named, the red-leaf is probably due to disturbances in the balance between absorption of water by the roots and transpiration. It is possible that the disease may be checked to a considerable extent by increasing the fertility of the soil so as to render the plants more resistant. The shrivel is found mostly among the white grapes and is also due to disturbed nutrition or deficiency of water supply. The root-rot is similar to if not identical with the disease described in French literature as *pourridie*. It is caused by one or perhaps several fungi not yet fully determined. It often kills the vines in a single season but it may only kill the vines after three or more years.—E. MEAD WILCOX.

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[29] KUNZE, GUSTAV., Ueber Säureausscheidung bei Wurzeln und Pilzhyphen und ihre Bedeutung. Jahrb. Wiss. Bot. **42**:357–393. 1906.

[30] SIMMONS, H. G., Remarks about the relations of the floras of the Northern Atlantic, the Polar Sea, and the Northern Pacific. Beih. Bot. Centralbl. **19**:149–194. 1905.

[31] BUTLER, O., Observations on some vine diseases in Sonoma county, California. Bull. Calif. Exp. Stat. 168:1–29. *1 pl. figs. 1–5*. 1905.

Preserving plants.—POLLACCI, speaking before the Italian Botanical Society,[32] commends his method, proposed in 1900, for preserving plants in a watery solution of sulfur dioxid. Specimens so preserved in 1900 have retained perfectly their form and are in as good condition for sectioning as when fresh. He has improved the method of making the solution and has devised a means of retaining perfectly the green color. To make the solution, place sodium bisulfid in a large flask, add sulfuric acid drop by drop, and conduct the gaseous SO_2 through water, which quickly becomes saturated and may be preserved for use as needed. To retain green color immerse the material in a 1 per cent. watery solution of copper sulfate, leaving it 24 to 48 hours according to the consistence of the tissues; then transfer to the preservative solution.—C. R. B.

Teratology in Salix.[33]—MOTT records various cases of teratology in the flowers of two Californian willows, *S. lasiandra* Benth. and a hybrid of *S. lasiandra* Benth. and *S. babylonica* L. In making the statement that no mention has been made for Salix of an intimate association of microsporangial and megasporangial tissue he overlooks an earlier account by the reviewer,[34] who described and figured equally intimate associations. According to MOTT, the abnormalities indicate that the ancestral Salix flower consisted of a pistil and two stamens with a four-parted perianth, the present unisexual condition having been reached by the suppression of the organs of one sex. Hybridization seems to offer the most likely explanation of the abnormalities.—CHARLES J. CHAMBERLAIN.

Nectaries of Cruciferae.—VILLANI has made an exhaustive comparative study of the nectaries of Cruciferae[35] and concludes that on the basis of their number the Cruciferae can be divided into four types, and on the basis of their position into generic groups. The diverse forms as to nectaries are referable to one primitive type, having four nectaries, two of which are at the base and external to each stamen, constituting an external dimerous cycle, and two at the base and between each member of each pair of long stamens, constituting an internal dimerous cycle. The tetramery of the corolla is only apparent, the whole flower being purely dimerous. The nectaries function both for securing cross pollination and protection.—C. R. B.

Morphology of Chloranthus.—Miss HELEN M. ARMOUR[36] has published the results of her study of Chloranthus, especially interesting as extending our

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[32] POLLACCI, G., Nuovo metodo per la conservazione di organi vegetali. Bull. Soc. Bot. Ital. **1905**:242.

[33] MOTT, WILLIAM WARNER, Teratology in the flowers of two Californian willows. Univ. Cal. Publ. Bot. **2**:181–226. *pls. 19–20.* 1905.

[34] BOT. GAZ. **23**:147–179. *pls. 12–17.* 1897.

[35] VILLANI, A., Dei nettarii delle Crocifere e del loro valore morfologico nella simmetria florale. Malpighia **19**:399–439. 1906.

[36] ARMOUR, HELEN M., On the morphology of Chloranthus. New Phytol. **5**:49–55. *pls. 3–4.* 1906.

knowledge among the more primitive Archichlamydeae. The development of the floral parts and both lines of sporogenesis were studied, the general conclusion being reached that the characters agree with those of the majority of the primitive Archichlamydeae. In the ovule the sporogenous tissue develops as a mass of cells, from which usually a single mother-cell is selected, a late differentiation of that cell which is quite characteristic of gymnosperms. The linear tetrad is formed, but there is the greatest irregularity in the selection of the functioning spore.—J. M. C.

Embryo of Symplocarpus.—C. O. ROSENDAHL[37] has made a preliminary announcement of a remarkable feature of the embryogeny of Symplocarpus. The endosperm destroys both integuments and pushes into the basal tissue of the ovule. There is a short, thick suspensor, and at this end of the "protocorm" both hypocotyl and plumule are differentiated. The developing embryo destroys the endosperm and all of the ovular tissue except at the very base, and thus comes to lie free in the ovary cavity! This means that the "seeds" of Symplocarpus are naked embryos. The full paper, with illustrations, will be looked for with interest.—J. M. C.

Nucleoli in mitosis.—The nucleoli in the vegetative cells of *Equisetum arvense*, according to G. BARGAGLI-PETRUCCI, play an important part in mitosis.[38] In the resting nucleus there is a single centrally placed nucleolus. At the beginning of mitosis, the nucleolus divides, one of the daughter nucleoli passing to each pole of the nucleus, where it perforates the nuclear membrane and passes to the apex of the achromatic figure.

While the figures are doubtless accurate, much more evidence will be required to establish the contention that the nucleoli play such a rôle in mitosis.—CHARLES J. CHAMBERLAIN.

Resistant potatoes.—The selection of races resistant to disease is one of the most promising methods of meeting many kinds of plant diseases. The resistance of Irish potatoes to blight, tuber rot, and scab has been worked out in an admirable way by STEWART.[39] More than one hundred and fifty varieties were tested and their difference in susceptibility is graphically represented. In general, varieties having strong, woody, moderately branched, upright stems, and medium sized, rather thick, firm, hairy leaves were more resistant than varieties possessing weak, much branched, decumbent stems with large, thin, smooth leaves.—F. L. STEVENS.

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[37] ROSENDAHL, C. OTTO, Preliminary note on the embryogeny of *Symplocarpus foetidus* Salisb. Science N. S. **23**: 590. 1906.

[38] BARGAGLI-PETRUCCI, G., I nucleoli durante la cariocinesi nelle cellule meristematiche di *Equisetum arvense*. Nuovo Giorn. Bot. Ital. **12**: 699–708. *pl. 2.* 1905.

[39] STEWART, W., Disease resistance of potatoes. Vermont Agr. Exp. Sta. Bull. 122. 1906.

Glycogen and paraglycogen.—A posthumous paper on this subject by Prof. LÉO ERRERA[40] has been edited from his notes by Dr. J. MASSART. It contains observations on the wide-spread occurrence of one or other of these bodies among the fungi, and their sparse distribution, perhaps only less observed, among the lower plants and animals, and possibly even among sea weeds and seed plants. ERRERA had interested himself in this reserve food for many years and had accumulated a great mass of bibliographical notes on it, which have been reduced to order and herein published.

Epidermal gaps.—Years ago MILDE and KNY and THOMAE described the occurrence of interstitial gaps in the winged bases of the leaves of three Osmundas and a Todea, and similar gaps have been found in the epidermis of floral leaves. Now LEIBLINGER,[41] incidentally, in the course of some cytological studies, finds such gaps in the epidermis of the scales of *Alluim Cepa*, which seem probably connected with the secretion of mucilage.—C. R. B.

Germination of moss spores.—TREBOUX contributes testimony upon the controverted question of the necessity of light for the germination of moss spores.[42] He finds twenty mosses of the most diverse families and three liverworts, a much larger number than has ever been tested before, able to germinate without light and (contrary to HEALD'S results) without cane sugar to replace its stimulating action.—C. R. B.

Haustoria of Osyris.—PIZZONI has published[43] an extended account of the haustoria of *Osyris alba*, supplementing the note of FRAYSSE[44] which unexpectedly forestalled PIZZONI'S paper after all his observations had been completed. He treats of the structure, relations to host, contents, duration, and dimensions of the haustoria.—C. R. B.

Nitrogen for maize.—SOAVE[45] finds that nitrogen supplied to maize in ammonium nitrate does not need to undergo nitrification in order to be available, so that, other things being equal, this compound of nitrogen is to be preferred to sodium nitrate, there being no delay in assimilation as affirmed by GERLACH and VOGEL.—C. R. B.

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[40] ERRERA, L., Glycogène et "paraglycogène" chez les végétaux. Recueil de l'Inst. bot. (Bruxelles) 1:343–379. 1905.

[41] LEIBLINGER, G., Ueber interstitienartige Strukturen in der pflanzlichen Epidermis. Ber. Deutsch. Bot. Gesells. **23**:387–396. *pl. 17*. 1905.

[42] TREBOUX, O., Die Keimung der Moossporen in ihrer Beziehung zum Lichte. Ber. Deutsch. Bot. Gesells. **23**:397–401. 1905.

[43] PIZZONI, P., Contribuzione alla conoscenza degli austorî dell' *Osyris alba*. Annali di Bot. **4**:79–98. *pl 3*. 1906.

[44] FRAYSSE, A., Sur la biologie et l'anatomie des suçoirs de l' *Osyris alba*. Compt. Rend. Acad. Sci. Paris **140**:270–1. 1905.

[45] SOAVE, M., L'azoto ammoniacale e l'azoto nitrico nello sviluppo del mais. Annali di Bot. **4**:99–114. 1906.

NEWS.

DR. A. F. BLAKESLEE has been appointed recently upon the botanical staff of the Philippine Commission.

PROFESSOR JOHN M. COULTER has been elected an honorary member of the Royal Botanical Society of Edinburgh.

DR. A. B. RENDLE has been appointed Keeper of the Department of Botany in the British Museum (Natural History).

DR. C. F. MILLSPAUGH, Field Columbian Museum, is spending three months in Europe, chiefly in the investigation of museums.

PROFESSOR DR. FRIDIANO CAVARA has been appointed director of the Botanical Garden of Naples in succession to the late Professor DELPINO.

MR. W. R. MAXON, U. S. National Herbarium, has just spent two months in Costa Rica collecting plants for the New York Botanical Garden.

DR. F. ROSEN has been advanced to the position of professor of botany and director of the Institute for Plant Physiology at the University of Breslau.

THE INFORMATION is just published that during 1903 there were 1,352,548 visitors at Kew Gardens; and during 1904 the number increased to 1,579,666.

THE ACADEMY OF SCIENCES in Munich has made a grant of *M*2500 to Dr. ROSZ, curator of the Botanical Museum, for zoological and botanical investigation in Central America.

DURING HIS PRESENT VISIT to the United States, Professor HUGO DE VRIES will deliver lectures at the summer session of the University of California and also at the University of Chicago.

AT THE RECENT Franklin Bicentenary at Philadelphia, the University of Pennsylvania conferred the degree of doctor of laws upon Professor HUGO DEVRIES, who was present to deliver an address.

DR. LESTER F. WARD, for twenty-five years the paleobotanist of the United States Geological Survey, has left the ranks of professional botanists by accepting the position of professor of sociology in Brown University.

IN THE RECENT DISASTER at San Francisco the building of the California Academy of Sciences was destroyed, containing the very valuable collection of Californian plants. It is reported that the types were saved by the heroic efforts of Miss ALICE EASTWOOD.

DR. E. N. TRANSEAU, Alma College, Michigan, has been appointed a member of the staff of the Station for Experimental Evolution at Cold Spring Harbor. He will work at evolutionary problems from the ecological side.

MISS FREDA DETMERS, formerly botanist to the Ohio Experiment Station, lately teacher of botany in the Columbus North High School, has been appointed instructor in botany in the Ohio State University and has already assumed the duties of that position.

A BRIEF biography of the late Professor LÉO ERRERA has been distributed, containing, in addition to an appreciative notice of his life and work, a fine likeness in photogravure, and a bibliography numbering 168 titles—a marvelous output, considering its high quality, for a man only 47 years old.

DR. D. S. JOHNSON, Johns Hopkins University, is spending April and May in Jamaica at the Cinchona station of the New York Botanical Garden. Dr. FORREST SHREVE, of the same university, is spending a year at the same station in work on the physiology and ecology of the forest of the Blue Mountains.

DR. VON DER CRONE, assistant in the Botanical Institute of the Royal Agricultural College at Poppelsdorf, near Bonn, returned from a journey in Spain ill with typhus, from which he died on the 23d of February last. He had already published some recent studies on a cause of chlorosis and was prosecuting further work in chemical physiology.

THE EDITOR of *Flora and Sylva*, having issued three volumes in serial form at less than cost, has become convinced that it does not appeal to a sufficient number of readers to justify its continuance as a monthly magazine. Hereafter it is to appear as a yearly volume, but otherwise precisely as heretofore. The next volume will appear in the autumn.

THE MARINE BIOLOGICAL STATION of the University of Washington, which is located at Friday Harbor, Washington, will open for its next season June 25 and will close August 5. The station is supplied with a steamer for transportation and deep dredging and offers good advantages for biological work, together with the pleasures of camping and tramping. Professor BRUCE FINK, Iowa College, will have charge of the botany.

PROFESSOR HUGO DE VRIES opened the course of spring lectures at the New York Botanical Garden on April 21st by an address upon "The correlation of characters in plants." Other lectures in this course will be given by W. A. MURRILL, ARTHUR HOLLICK, L. M. UNDERWOOD, C. S. GAGER, MARSHALL A. HOWE, G. V. NASH, H. H. RUSBY, D. T. MACDOUGAL, and N. L. BRITTON.

INSTRUCTORS in botany at the Biological Laboratory of the Brooklyn Institute located at Cold Spring Harbor, Long Island, for the summer of 1906 are Dr. D. S. JOHNSON, in charge of cryptogamic botany, Dr. E. N. TRANSEAU, in charge of plant ecology, and Mr. H. H. YORK, associate in botany. The laboratory will be open during July and August, the courses beginning July 5, and continuing six weeks.

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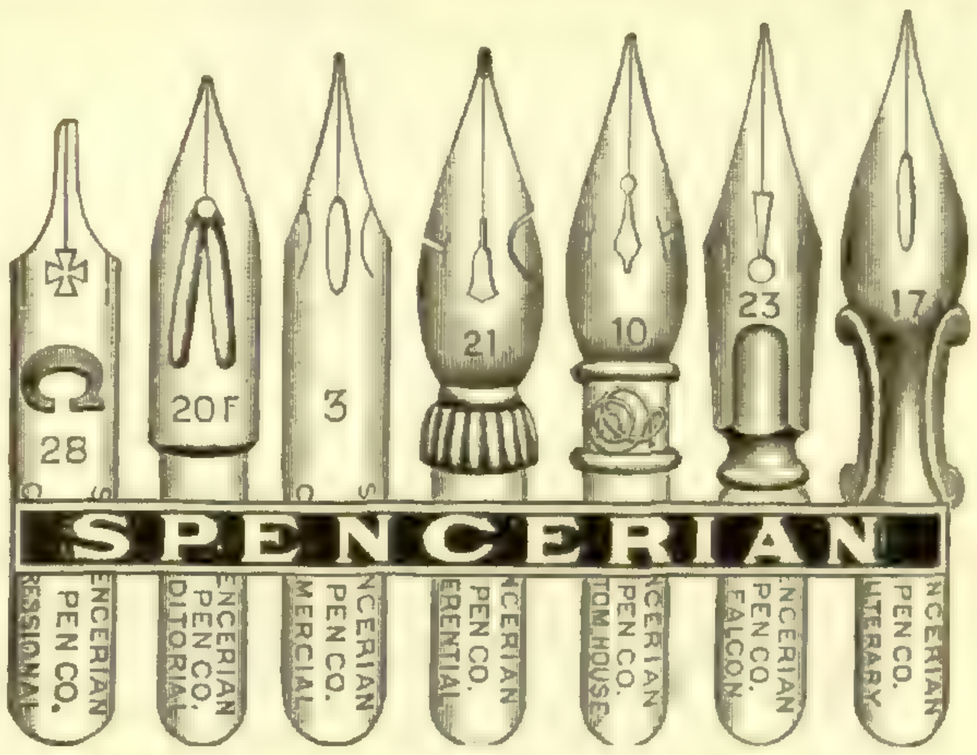

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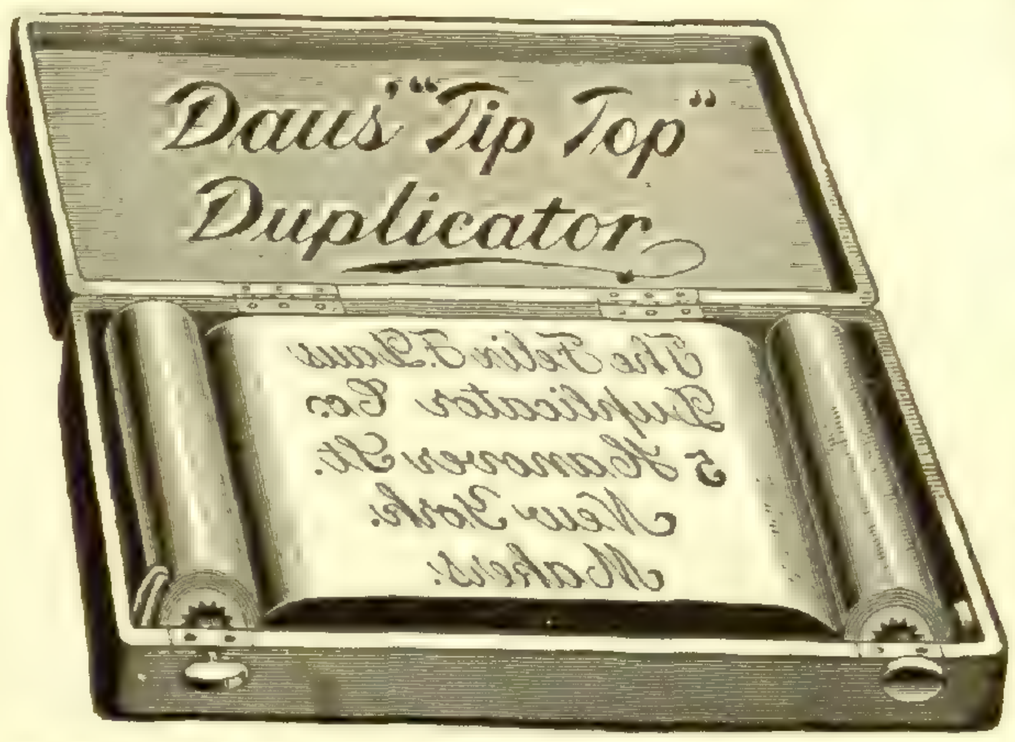

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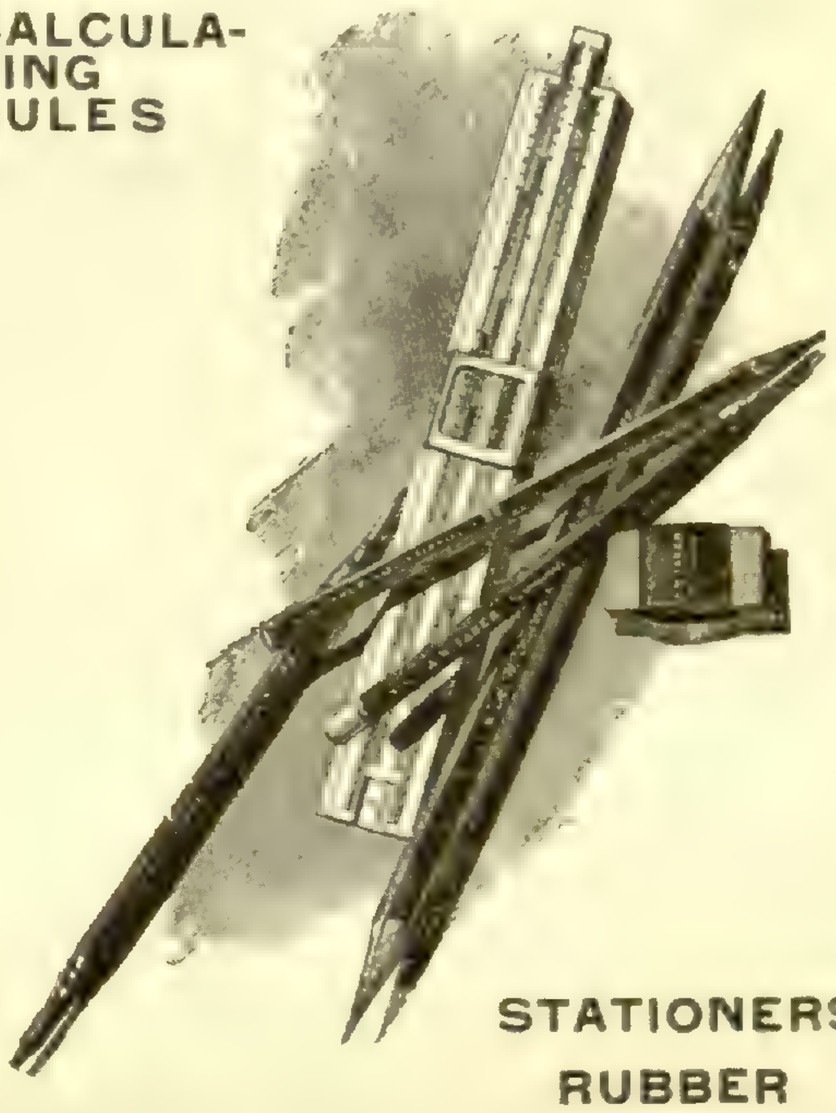

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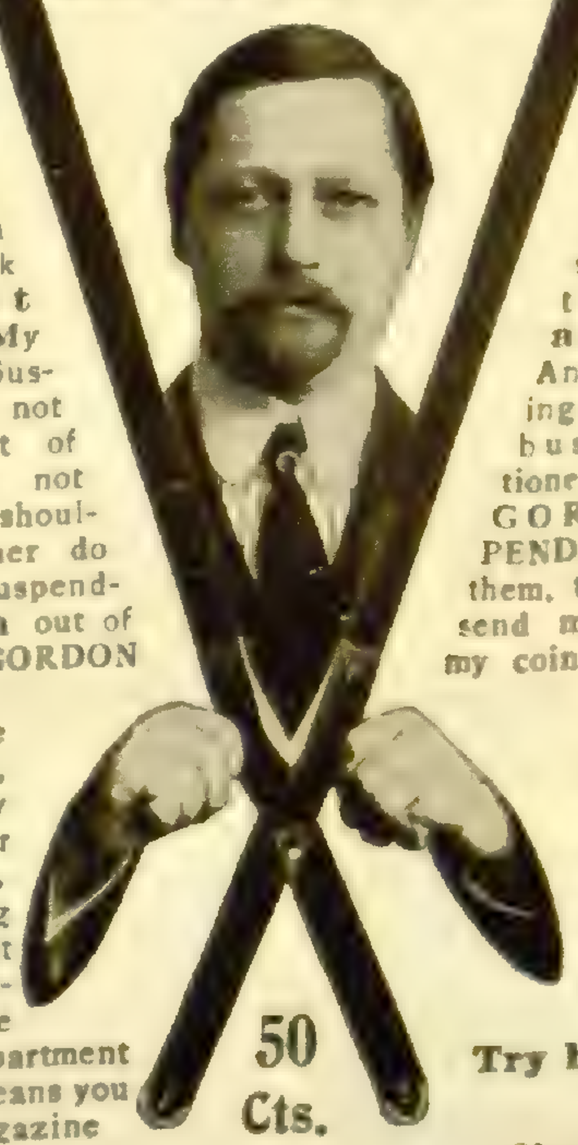

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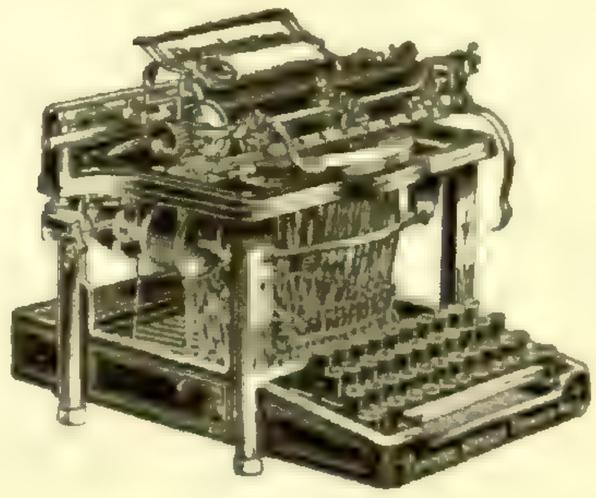

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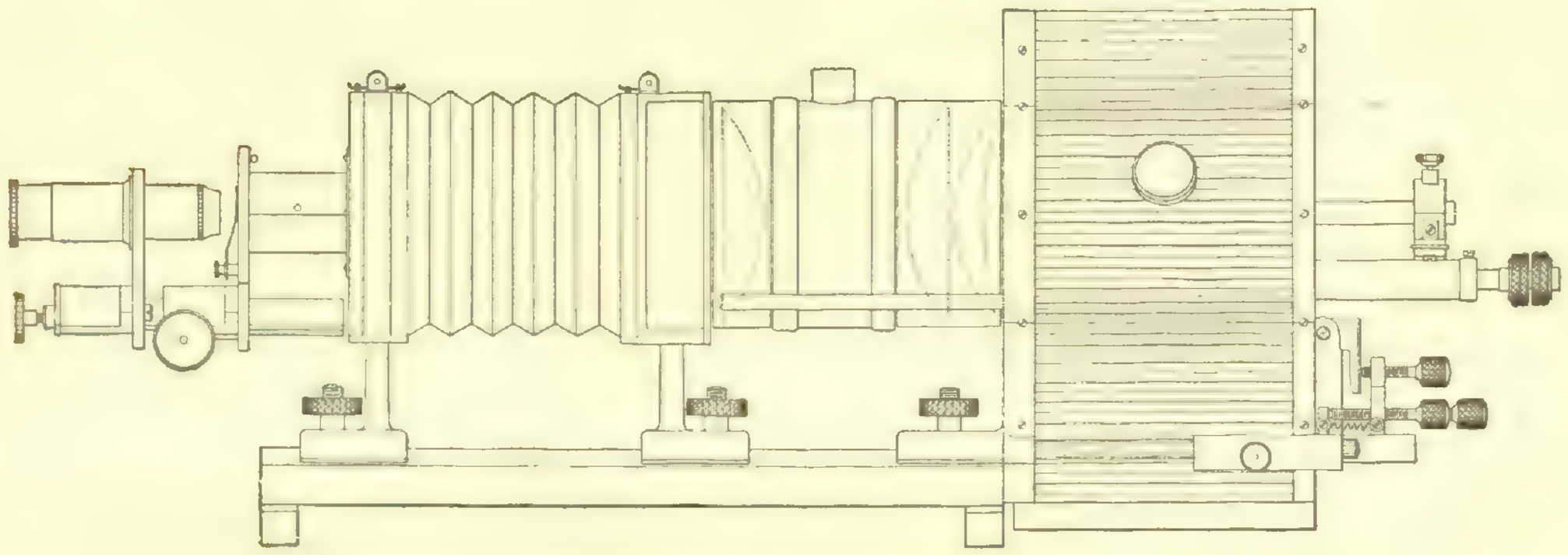

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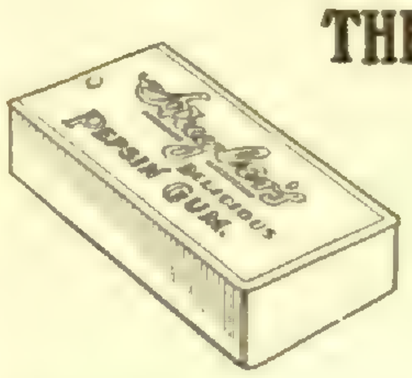

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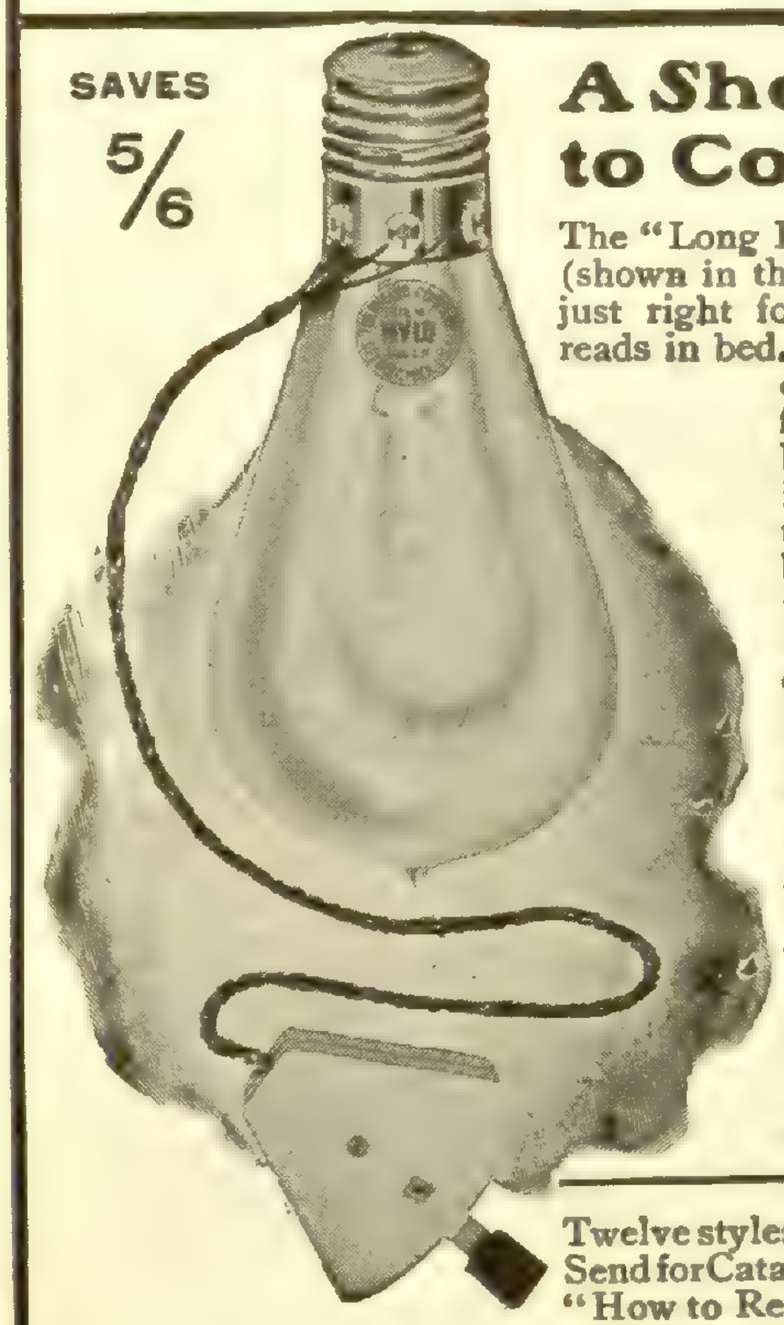

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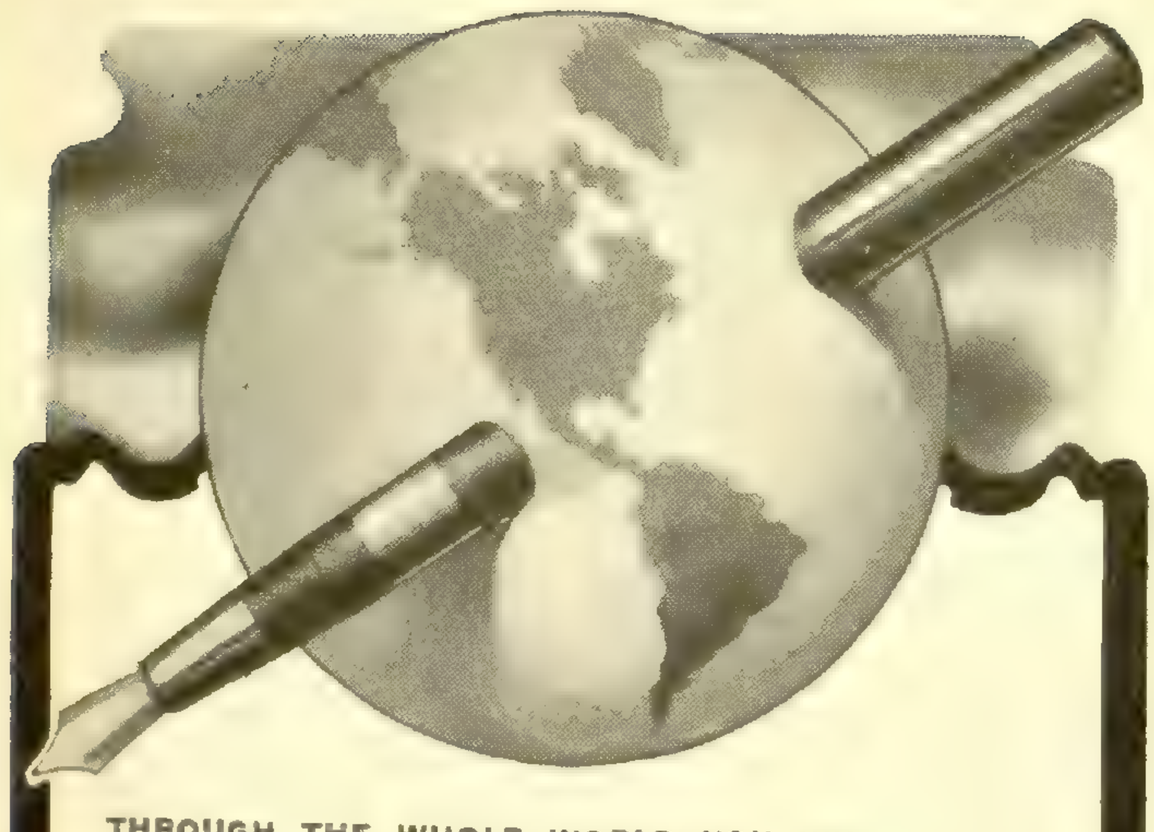

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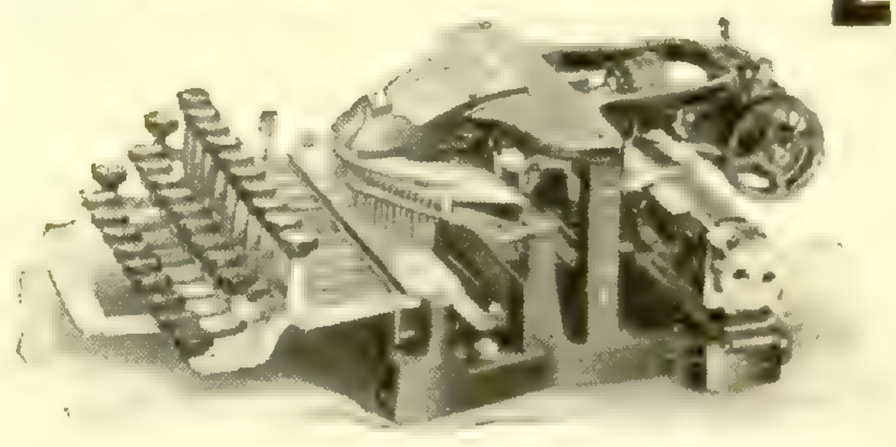

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BOTANICAL GAZETTE

JUNE, 1906

SOME STUDIES REGARDING THE BIOLOGY OF BUDS AND TWIGS IN WINTER.[1]

KARL M. WIEGAND.

(WITH EIGHT FIGURES)

DURING the winter months in temperate and arctic climates, the meristematic tissues of shrubs and trees assume a more or less completely dormant or resting condition, and become separated from the surrounding atmosphere by tissues of varying thickness and varying degrees of resistance to the passage of water vapor. A detailed study of these structures during the cold period has brought out many interesting facts ordinarily escaping casual observation.

In the twigs the cells of the cambium lie close together without intercellular spaces, but the cortical cells usually do not touch at the corners, and consequently in the cortex there is a more or less elaborate system of intercellular spaces. The main structural protective measure seems to be the firm epidermal layer with heavily cutinized outer wall, which is always present at this period.

There were no stomates on the twigs in any of the species I examined. Gas diffusion takes place mainly through the lenticels; but perhaps to a slight extent also through the cuticle itself. All the living cells contain a large amount of water, 51–55% in most fruit trees, 63% in Forsythia, and the quantity in each species is remarkably constant, rarely varying more than four to five per cent., and usually even much less.

Regarding the time during the previous summer when the bud fundament is first distinguishable, ALBERT[2] found that, out of

[1] Contributions from the Department of Botany of Cornell University. No. 105.

[2] ALBERT, P., Beiträge zur Entwickelungsgeschichte der Knospen einiger Laubhölzer. Forstlich-naturw. Zeitschr. 3: 345, 393. 1894.

15 species of trees bearing scaly buds, the first leaf fundament in one (*Betula alba*) was present as early as May, in three at the beginning of June, in eight at the beginning of July, in two August 1, and in one not until September. The flowers were always formed later than the leaves. Some of the naked buds he found to start early in the previous season (Elaeagnus, Cornus); others, as for instance *Robinia Pseudacacia*, did not start until the spring of the year in which they were to unfold. He found that in general the buds were further progressed at the beginning of the winter the farther north the plants were native.

BEHRENS[3] found that in fruit trees the flowers are first distinguishable at a later date, as for example, in the cherry during July, and in the pear about August 11.

My own observations lead me to believe that in many cases the fundaments are present quite early. The buds of the peach were well formed July 15, and small buds were evident in the leaf-axils of forest trees as early as June 1. This suggests that, in some cases at least, the bud fundament may be present as early as the unfolding of the previous winter's buds.

Those that start quite early have usually reached an advanced stage in development by the time cold weather overtakes them in the fall. The rudimentary flower or shoot for the next season, together with all its organs, is present in the buds of some species, as for instance in the horsechestnut; while in others a varying number of nodes and internodes are thus stored. To inclose so elaborate a structure a certain number of leaves have been modified into scales which closely overlap, or are firmly cemented together at their edges around the young shoot. Such buds are found especially upon trees and shrubs with definite annual growth. The scales are usually composed of several layers of parenchymatous cells with intercellular spaces, moderately firm and slightly cutinized epidermis, on the inner side, and a very strong heavily cutinized outer epidermis, usually supported by mechanical tissue of varying amount beneath.[4] The parenchymatous cells of all or

3 BEHRENS, J., Entwickelung und Bau der Blütenknospen unserer Obstbäume und Obststräucher. Gartenflora **47**:269. 1898.

4 For published descriptions of bud structure see: BEHRENS, J., *l. c.*; FEIST, A., Ueber die Schutzeinrichtungen der Laubknospen dicotyler Laubbäume während

all but the outermost scales are living and contain a large amount of water throughout the winter. The inner scales are frequently almost destitute of epidermal thickenings and are quite green and fresh. Because of the much larger size of the cells in the scales, and much larger vacuoles, there is much more water present in these structures than in the young shoot whose cells are small and nearly filled with protoplasm. This it will be seen is an important consideration when the buds freeze during the winter. The absolute amount of water in the whole bud is however very nearly the same as that in the young bark, being about 51 to 55% for the fruit buds examined; and, as in the bark, this amount is remarkably constant for the species.

The proportion of space occupied by the young shoot varies with the species and nature of the bud. In flower buds this proportion is usually greater than in leaf buds. In many cases only a very small fraction of the total volume is shoot-tissue, all the rest being composed of scales; but in other cases, as for instance the flower buds of pine, almost the whole volume is made up of cones, leaves, and stem; while the scales are very thin, dry, and firmly cemented together. In this case of course nearly all the water is located within the young shoot. The spaces between the various organs and scales usually contain air alone; but in some cases, as for example in apple and horsechestnut, there is also a large amount of wool present in which the organs are seemingly imbedded. In Populus and some other trees the spaces are more or less completely filled with resin.

Buds of most indefinite growers differ from those of the majority of definite growing trees in two essential ways: in the slight development of the fundament, and in the usual absence of scales. The young shoot is most frequently represented merely by a growing

ihrer Entwickelung. Nova Act. Leop. Carol. Ak. Naturf. **2**: 303–344, 1887, Ref. Bot. Centralb. **36**:43. 1888; SCHUMANN, C. R. G., Anatomische Studien über die Knospenschuppen von Coniferen und dicotylen Holzgewächsen. Biblioth. Botan. 15:32. Cassel, 1889, Ref. Bot. Centralb. **42**:275. 1890; GRÜSS, J., Beiträge zur Biologie der Knospen. Jahrb. Wiss. Bot. **23**:637. 1892; LUBBOCK, J., On buds and stipules. Jour. Linn. Soc. **30**:463–532. 1895; **33**:202–269. 1897; CADURA, R., Physiologische Anatomie der Knospendecken dicotyler Laubbäume. Breslau, 1887, pp. 42; MIKOSCH, K., Beiträge zur Anatomie und Morphologie der Knospendecken dicotyler Holzgewächse. Sitz. König. Akad. Wiss. Wien Math. Wiss. Kl. **74**[1]:723–755. 1877.

point without well-developed lateral organs, and can therefore be protected more economically by being sunk in a pit produced by a ring-like growth of cortex and cork, as is commonly the case. This pit is then closed at the mouth by an ingrowth of the cork itself, as in Gleditschia, or by a feltlike mass of hair, as in Robinia and other species.

In the case of the large buds with the shoot considerably advanced in growth, the bud-scale method seems the only feasible way of covering them. Another advantage in this method lies in the telescopic expansion of which scaly buds are capable early in the season while unfolding. Growth is thus permitted, but at the same time the protective qualities are not lost. In the maples and horse-chestnut the tube formed by the enlarged scales often reaches the length of 2 to 8^{cm}. By this means buds may open early in the spring and still be protected from excessive transpiration. Scaleless buds usually remain nearly dormant until later in the spring when the weather conditions are not so severe.

PHYSICAL PHENOMENA OF BUDS AND TWIGS WHEN NOT FROZEN.

The scaleless buds of the indefinite growing trees and shrubs grow very little before or during the winter. In the autumn the very limited growth is soon stopped by the advent of cold weather, and from this time until late spring scarcely any change can be detected. With the scaly buds however it is otherwise. From the inception of the fundament in July or June until cold weather there is a very considerable growth resulting in the buds of various sizes found upon the different species of trees during the winter. Little accurate work has been done towards determining the characteristics of this growth, but the results obtained in our laboratory by W. M. MORGAN during the fall of 1901 seem to show that in the case of fruit trees the growth is very uniform and gradual up to about November 15. In some cases slight fluctuations occurred which could not be accounted for, and in one or two instances these seemed periodic; but on the whole there appeared neither acceleration nor retardation until the time mentioned, when the increase in size ceased quite abruptly. From the middle of November until March 1 there was no growth in peach buds, the curve remaining almost exactly horizontal and fluctuating very little. On March 23,

several days of warm weather occurring, the peach buds began to grow rapidly and uniformly until April 23, one month later, when they came into flower. With the apple and apricot the results were very much the same. Growth almost ceased November 15, and from this time until March 1 the increase was apparent but exceedingly slight, amounting to only ½ to 1%. Renewal of activity began March 1, and from this time until April 23, seven weeks later, when the apricots flowered, and eight weeks later, when apple buds opened, the growth was very rapid. The curve after growth began was not so gradual as in the peach, but became much accelerated just before the flowers appeared.

Mr. MORGAN'S observations were as a matter of fact quite extensive, but only the above summary can be given here. At intervals of one week through the fall, winter, and spring, buds were taken from the same tree and as nearly as possible from shoots of the same vigor, a large number were measured, and the average taken as representing the size at that time. It was found impracticable to measure the same bud at different times, owing to the difficulty of manipulating the micrometer out of doors on very cold days, as well as to the fact that the measurements were liable to be taken at different temperatures each time. A Zeiss cover-glass measurer was found the most convenient instrument for the work. From the tables thus made a great many curves were plotted representing the changes in various fruit trees. The results, however, agreed very well, and in the peach, apricot, and apple were as stated above.

From these careful observations, therefore, contrary to the general belief, it seems that fruit buds at least do not grow to any extent in winter. Their swelling period is confined in the north to a few weeks just previous to the opening of the bud. Regarding our forest trees and shrubs no accurate work seems to have been done toward the determination of their curve of growth. From casual observations, I am inclined to believe that a majority will be found to agree with the fruit buds. This seems to be true of the sugar maple, whose buds are practically as large in November as in early March, also of the ash, oak, etc. On the other hand, the buds of a few plants, as, for instance, *Salix discolor*, *Ulmus fulva*, and *Ulmus scabra*, seem to increase in size early in February. However, actual measurements are necessary to determine these points.

KÜSTER[5] found that during a specially mild winter the buds of maple did show a very slight growth both in the lateral organs and in the young axis. No new organs were started either in maple or other species examined, except rarely in *Alnus cordifolia*.

ALBERT[6] found that practically all buds became dormant soon after leaf-fall until spring again. The first change in spring was a stretching of the tissues, further development of the parts taking place only later.

It is a known fact in physics that the amount of heat absorption varies, among other factors, with the color of the body investigated. In other words, the same body if colored differently will absorb a varying amount of heat from a constant source, depending upon the color. Winter buds and branches are in many cases highly colored, and the question naturally arises as to how this affects the heat absorption of the bud during the winter and spring months.

Regarding the extent to which color will affect heat absorption, in addition to the records in works on physics, the experiments of WHITTEN[7] are interesting. He found that thermometer bulbs wrapped in muslin of different colors, green, purple, black, and white, or with pieces of muslin of these various colors spread over them, or with the bulbs coated with a wash of similar colors, showed a marked difference in reading when exposed to bright sunlight. The average difference between the black- and white-washed bulbs was 16°, between the white and purple 15°, and between the white and green 13°. At one time a difference of as much as 21° between the white and purple bulbs was found.

However, the actual experiments with buds have been rather few and the results are not so definite as one might wish. The most elaborate were those of WHITTEN described in the above-cited report. He selected a row of peach trees containing several varieties, and whitewashed them during the winter. During warm days of the unusually changeable winter the unwhitened buds swelled considerably, and during subsequent cold spells most of

[5] KÜSTER, E., Ueber das Wachsthum der Knospen während des Winters. Beitr. Wiss. Bot. (FÜNFSTÜCK) 2:401. 1898.

[6] ALBERT, P., Beiträge zur Entwickelungsgeschichte der Knospen einiger Laubhölzer. Forstl.-naturw. Zeitschr. 3:345–376, 393–419. 1894.

[7] WHITTEN, J. C., Winter protection of the peach. Bull. 38. Missouri Agric. Exp. Station, April 1897.

them were killed. The unwhitened buds swelled and grew perceptibly before any swelling could be detected in those that were whitened. The difference in size March 20 was plainly shown in drawings of the sections of the two classes of buds. Whitened trees came into bloom about one day later than unwhitened trees of the same variety. In 1896–97, owing to a more moderate spring, the difference in time of flowering was still greater. The whitened buds of each variety opened two to six days later than those that were not whitewashed.

The differences in the actual time of flowering, however, does not express the difference in time of the swelling of the buds. The whitewashed buds did not begin to swell until almost time for the flowers of normal trees to appear, while the unwhitened ones began to swell three or four weeks earlier, as shown by the drawings above mentioned.

These experiments of WHITTEN seem to show that in the peach, at least, the dark-purple color of the buds tends to cause earlier activity in the spring, accompanied by earlier swelling and flowering. The only doubt, it seems to me, lies in the effect of the whitewash upon the growing tissue. As mentioned below, some non-porous substances seem to retard respiration perhaps to such an extent that growth also is retarded, but the whitewash would seem porous enough to escape this criticism. In a more recent paper WHITTEN[8] has shown that the temperature within whitened and unwhitened twigs differs by several degrees. In bright sunlight the difference was as much as 15° C., the unwhitened being the warmer. The whitened twigs were nearly of the same temperature as the atmosphere.

WHITTEN[9] has also shown that purple peach twigs transpire considerably more than green ones. This was probably due to the greater temperature and is probably an additional factor in the winter-killing of the peach.

[8] WHITTEN, J. C., Preventing frost injuries by whitening. Pacific Rural Press **60**:276. 1900.

[9] WHITTEN, J. C., Das Verhältnis der Farbe zur Tötung von Pfirsichknospen durch Winterfrost. Inaug. Diss. Halle. 1902. p. 35. See also in this connection MACOUN, W. T., Some results of experiments in spraying, etc. (whitewashing to retard bud development.) Ontario Fruit Growers Ass. Rep. **1899**:100, and "Experimental Farms," Canada, **1899**:92.

Wishing to determine the effect of natural color and surface of buds upon the absorption of heat, I carried through several experiments with horsechestnut buds, which gave some interesting results as follows.

On February 8, in bright sunshine, a large horsechestnut bud was obtained and the scales dissected away, care being taken that they were not unnecessarily injured. Two thermometers previously tested as to accuracy were obtained, and over the bulb of one the bud scales were carefully imbricated and firmly held in place by a few turns of black thread. There was enough resin present to cement the scales firmly together and thus form an artificial horsechestnut bud with the thermometer bulb in place of the normal shoot. The instruments were then placed on a table out of doors and in the shade where they were allowed to lie. As soon as the readings were nearly the same,[10] the table and instruments were carried to a place in full sunshine, care being taken that the two bulbs projected about 6.5^{cm} beyond the edge of the table so as not to be affected by direct radiation from the surface of the latter. The readings were taken as follows:

TABLE I.

Horsechestnut bulb and naked bulb, from shade out of doors to sunlight. (See *fig. 1.*)

Naked bulb	Horsechestnut bulb	Time min. & sec.	° Difference	
32° F.	32° F.	0:00	0 ° F.	(0.0° C.)
33	32	0:10	1	0.5
34	33	0:30	1	0.5
34.5	34	1:10	0.5	0.2
35	35.7	1:30	0.7	0.4
36	37	2:00	1	0.5
36	38	2:30	2	1.0
37	39	3:00	2	1.0
37	40	3:30	3	1.6
37	42	4:00	5	2.8
38	44	4:30	6	3.3
38	45	5:10	7	3.9
38	48	6:00	10	5.5
38	49	7:00	11	6.1
38	50	10:00	12	6.6
39	51	14:00	12	6.6
39	52	19:00	13	7.2
39	53	24:00	14	7.7
39	54	29:00	15	8.3

[10] They could not be made to read the same because the overcooling point in the bud had been reached.

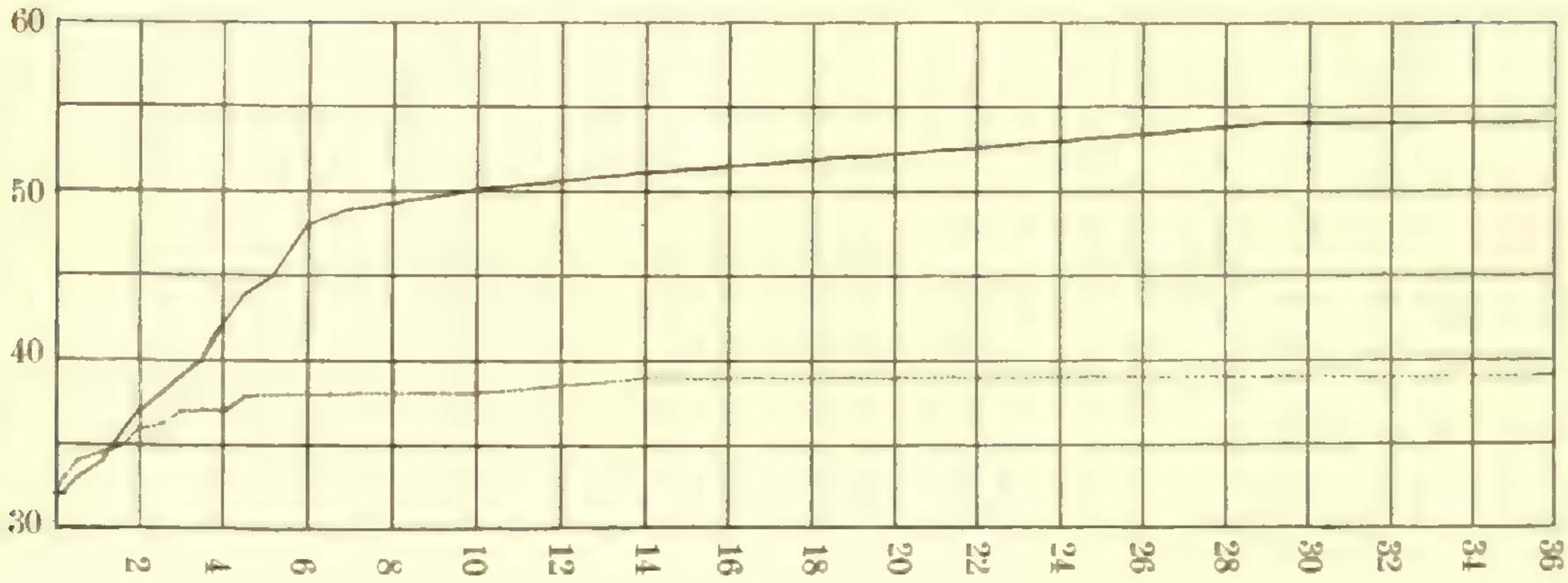

FIG. 1.———horsechestnut bulb; ..... naked bulb. Abscissas represent 5° F.; ordinates, 2 minutes. See Table I.

No further rise was noted, although the instruments remained in place more than an hour. The experiment was repeated several times during the spring, both with the same bud and with a fresh one, in every case with practically the same result, namely a much faster rise in the bud-bulb, amounting finally to an excess of 5 to 12° C. over the other bulb.

That the above differences were due mainly to the dark brown color of the bud seems probable from another series of experiments in which the naked thermometer-bulb was coated with brown drawing ink. Readings were taken under the same conditions as before. In these cases the difference was but slightly in favor of the horsechestnut bulb, probably because of the less highly polished surface of the ink bulb. It would seem, therefore, that the point is fairly well demonstrated that in the case of the horsechestnut, at least, the color does make considerable difference in the absorbing power of the bud as regards heat. Although no experiments were performed, there seems no reason why the same should not be true also of other dark-colored buds.

With these results in mind I made a few observations one spring to see if there was any relation between the color of the buds and the time of swelling and opening. It seemed reasonable to expect that the darker the bud, and consequently the more heat absorbed, the earlier the bud would swell and open in the spring; and all the more because this was the exact conclusion reached by WHITTEN with his peach experiments. Unfortunately, accurate record of the time of swelling and of opening were not kept; but still I believe

some important general tendencies can be made out from the notes, and therefore the following table is inserted:

Name	Color of buds	Time of opening
Magnolia acuminata	yellow-gray or olive	late
Ailanthus glandulosus	yellow-gray	late
Acer Negundo	pale, whitish	medium
Juglans cinerea	pale, grayish	very late
Quercus alba	gray	very late
Salix alba	yellow	early
Populus grandidentata	pale	very early
Syringa vulgaris	pale, yellowish	early
Betula lutea	olive	late
Fagus americana	olive	medium
Populus dilatata	yellow-brown	medium
Salix cordata	olive or purple	early
Salix Bebbiana	brown	medium
Salix sericea	brown-purple	early
Salix petiolaris	brown-purple	early
Acer Saccharum	bark olive-brown	medium
Tilia americana	red	medium
Aesculus Hippocastanum	dark brown	early
Acer saccharinum	red	very early
Prunus persica	purple	very early
Other species of Prunus and Pyrus	red, brown, or gray	early
Crataegus coccinea	red or dark olive	medium
Ulmus fulva	dark brown	early
Ulmus scabra	black	very early
Salix discolor	black	very early

It is not to be expected that the time of opening or even the time of swelling will in all cases be proportional to the color of the buds alone. The protoplasmic characteristics of the particular species or genus undoubtedly play a very important part; but bearing this in mind the following suggestions appear in the above table. Nearly all of the light-colored buds are also late to swell and open. None of the dark red or especially the black buds open late. On the other hand, a few light buds, as for example those of Syringa, Populus, and *Salix alba*, open quite early. This may be due to more easily aroused protoplasm than is present in most buds. Possibly if these buds were black they would open still earlier and therefore suffer injury from the frost; thus the present lighter color may serve as a means of protection.

During the spring of 1901, about March 1, some experiments were started to determine if possible whether other buds might be influenced by color in a way similar to the peach buds white-

washed by WHITTEN. Instead of whitewash, black paint was used to see if they might be made to open earlier. Two kinds of paint were prepared, one made of lamp black mixed with linseed oil, the other of lamp black and xylol. Buds and twigs of *Syringa vulgaris*, *Ailanthus glandulosus*, *Populus dilatata*, and apple were treated to a coat of oil paint; while some others of Syringa, Ailanthus, and apple were coated with xylol paint. The results were as follows:

Syringa.—Xylol-painted buds much behind the normal during vernation; they looked unhealthy, one or two being entirely dead. Oil-painted buds never began to swell, all dead.

Ailanthus.—The xylol paint made no difference with the killing back of the branches nor with the development of the buds. Oil paint prevented the swelling of the buds; they never opened.

Apple.—Xylol-painted buds much behind the others; one completely dead. Oil-painted ones all dead.

Populus dilatata.—Oil-painted buds showed much more rapid swelling than normal. When just opening the blackened buds were 6 to 8^{mm} longer.

These results are evidently in the main exactly opposite what we were led to expect. I suspect that the explanation lies in this, that the coating of the surface of the bud with paint prevented respiration, and thereby inhibited growth just as did varnish used on naked buds as described later in this paper, although it is possible that some toxic property of these substances might have had something to do with the matter. The xylol furnished a much more porous layer than did the oil, and the inhibition was therefore much less. The buds of *Populus dilatata* are normally almost completely infiltrated with resin in the spaces between the organs and on the surface, and consequently may have some other means of obtaining oxygen for respiration. The coating even with oil paint, therefore, did not injure them. On the contrary, the black color seemed to cause an acceleration in growth.

In concluding this part of the subject we may say that in the climate of New York, buds during the winter seem to remain in an almost dormant condition until a short time previous to their opening in the spring. In Missouri swelling of peach buds began much earlier than in New York.

Color through its power of absorbing heat seems to have some effect upon the growth of buds in the spring. Early buds are in

most cases dark, and artificial darkening, when unaccompanied by deleterious factors, seems to accelerate the opening.

PHYSICAL CONDITIONS IN FROZEN BUDS AND TWIGS.

From many inquiries it would seem that very few people are really sure whether free ice is actually present in buds in winter. Nevertheless, this is one of the most common phenomena connected with the winter condition of trees and shrubs. To put the matter on a firm basis of observation I undertook, during the winter of 1901, to section buds of various trees during cold pericds and to determine under the microscope the amount of ice present. The method employed was as follows. Early in the morning, at about sunrise, after a fall of temperature to −18° C. or below, a table, microscope, razor, needles, slides, and cover glasses were placed in a shady situation in the open air, where they were allowed to become thoroughly cooled. Free-hand cross sections of the various buds were then made, and mounted on the slide. For a mounting medium cedar-wood oil was found best. A small quantity of this in a vial was allowed to cool with the instruments. One important advantage in the use of cedar-wood oil over those of a denser nature lay in the fact that it did not congeal at the low temperatures of the experiment. The ice remained unmelted in the preparation and could be observed at leisure; or if the thawing process was under study the slide could be carried to a warm room and placed under another microscope. The melting ice was unable to evaporate from the section, and therefore it was easy to determine whether the water was all reabsorbed, and the approximate rate of absorption.

The ice was found to occur always in broad prismatic crystals arranged perpendicular to the excreting surface; and usually formed a single continuous layer throughout the mesophyll of the scale or leaf, to accommodate which the cells were often separated to a considerable distance (*figs. 2, 3, 4*). This ice sheet was composed of either one or two layers of the prismatic crystals, depending on the water content of the adjacent surfaces, and was often as thick as the whole normal scale. The cells surrounding the ice, having lost their water content, were in a more or less complete state of collapse,

depending upon the resistance of the walls, and often occupied a space smaller than the ice itself. These cells were uninjured, however, and would resume their normal condition on thawing. In all cases more ice was found in the scale than in the young shoot; never between the scales but always in the mesophyll. The cells of the embryonic shoot were so much smaller and their water content so much less, that frequently it was difficult to detect any ice forma-

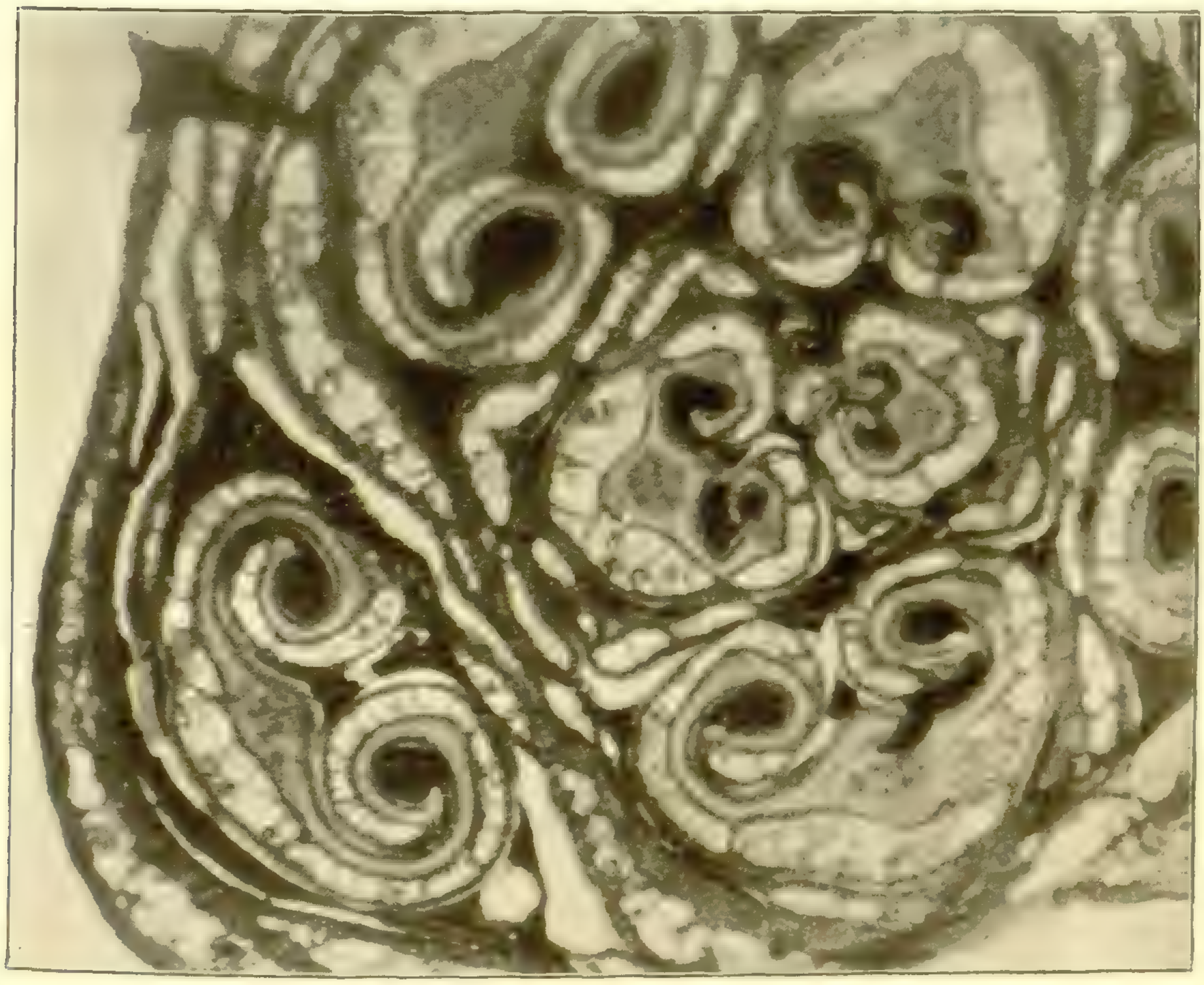

FIG. 2.—*Populus dilatata:* cross-section of bud, showing ice in bud-scales and foliage leaves.

tion whatever; but ordinarily very minute and numerous masses, at least, were scattered between the cells, and sometimes there were large masses such as appear in the outer organs. In young anthers the ice often filled almost the entire anther cavity, and in it the pollen grains were imbedded in a completely collapsed state.

The results of the observations regarding the occurrence of ice in buds may be summarized briefly as follows. The temperature was $-23.5°$ C. to $-18°$ C.

1. Tissue packed full of ice in shoot and in mesophyll of scales forming sheets parallel with the surface; rapidly and completely reabsorbed when the sections were thawed in oil. Sponging out of sections very marked.—*Populus dilatata* (*fig. 2*) and *P. candicans*, *Prunus serotina* and *P. virginiana*, *Betula lenta*, *Acer Negundo*, *Pyrus Malus* and *P. communis*, *Aesculus Hippocastanum*.

2. Containing a large amount of ice, but the water tardily reabsorbed on thawing in oil.—*Acer Saccharum*, *Tilia americana*, *Ulmus scabra*, *Crataegus punctata* (*fig. 3*).

FIG. 3.—*Crataegus punctata:* cross-section of bud, showing ice in bud-scales and floral parts.

3. No ice could be found at 0°C. Tissue dense, of small cells.—*Castanea dentata*, *Hamamelis virginiana*, *Fagus americana*, *Fraxinus americana*, *Juglans cinerea*, *Corylus rostrata*, *Quercus alba*, *Hicoria ovata*.

4. Other cases.—In *Pinus Strobus* and *P. sylvestris* there was a moderate amount of ice in the shoot and in the anther as well as in the inner scales. In *Syringa vulgaris* there was a very large quantity of ice in the scales and young shoot, especially in the anthers (*fig. 4*). In *Viburnum dentatum* and *Prunus persica* the amount of ice was small, but water was quickly reabsorbed.

Of the twenty-seven plants examined there were only eight that showed no ice in the buds at −18° C. These eight were sectioned later at −26.5° C., with the result that in Castanea, Hicoria, Fraxinus, and Juglans numerous minute ice crystals were found between the cells. It would seem, therefore, that ice may be found in all buds if the temperature is sufficiently low.

The accompanying illustrations are reproductions of photomicrographs taken by the writer during periods of low temperature.

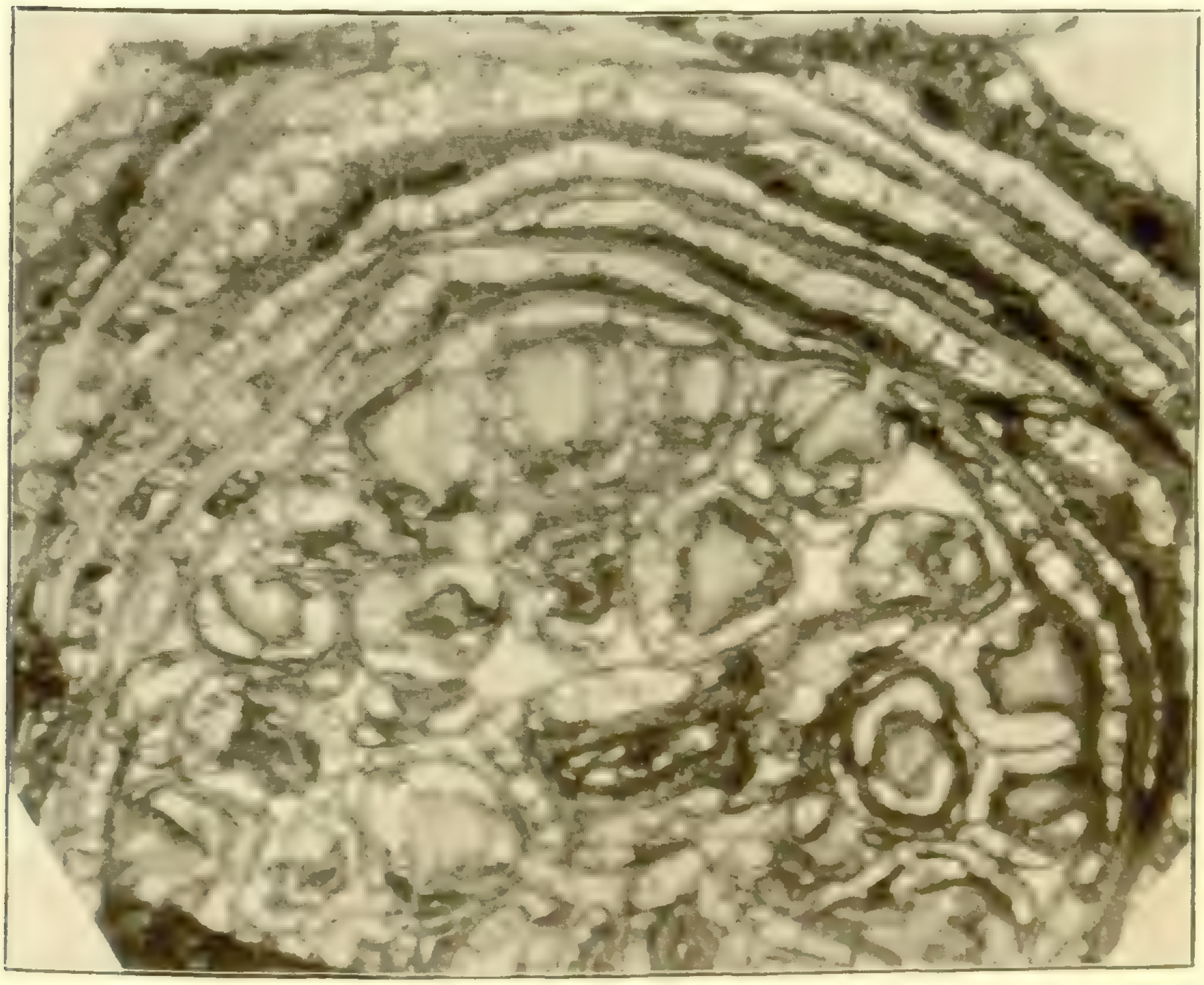

FIG. 4.—*Syringa vulgaris:* cross-section through flower bud while frozen; the light spaces filled with ice.

When the mercury registered at zero Fahrenheit or below, freehand sections mounted in oil as already described were photographed, the apparatus being set up in the open. The conditions for sectioning were so strenuous that very thin sections could not be obtained, and hence the rather poor quality of some of the photographs. The palisade-like ice prisms fill the light areas through the mesophyll of the scales and young leaves.

The question naturally arose as to the cause of the difference in ice content and why ice was absent in the eight species mentioned. Since lowering the temperature from −18° C. to −23.5° C. caused the appearance of ice in some, it would seem therefore to be simply a matter of temperature. But the degree of cold necessary to cause the separation of ice is proportional to the force which holds the water in the tissue. This in turn depends upon the relative proportion of water to cell-wall and protoplasm. We should expect, therefore, to find in those buds which are difficult to freeze a smaller amount of water than in other buds; also smaller cell-structures, since by this latter means the proportion of cell-wall and protoplasm is increased. When cells become smaller it is usually the water content that most rapidly diminishes, the protoplasm following at a much lower rate. I have made the following measurements of the cells and water content in seven of the species in which there was much ice, and in seven in which ice did not appear at −18° C.

	Max. aver. mm.	Min. aver. mm.	Text. of wall	% of water
A. Ice abundant in bud-scales, leaves, and growing point—				
Crataegus punctata	0.040	0.012	thin	49.4
Ulmus scabra	0.051	0.015	"	46.6
Syringa vulgaris	0.0045	0.009	"	53.2
Acer Negundo	0.021	0.009	"	45.9
Prunus serotina	0.021	0.015	"	47.6
Populus dilatata	0.025	0.018	"	39.3
Betula lutea	0.018	0.006	"	37.5
B. Ice not present at −18° C.				
Quercus alba	0.015	0.006	thick	22.7
Corylus rostrata	0.018	0.006	"	29.7
Castanea dentata	0.018	0.015	"	25.4
Fagus americana	0.008	0.003	"	26.8
Hicoria alba	0.048	0.015	very thick	31.4
Juglans cinerea	0.012	0.003	thick	25.0
Fraxinus americana	0.021	0.003	"	29.8

Our supposition regarding the smaller size of the cells and smaller water-content in the second group, therefore, seems to be upheld by these results.

In the twigs ice is also present in very cold weather, where it may be found in three different localities. The largest quantity occurs in the cortex, where the ice crystallizes in prisms arranged

in single or double series according to the law of freezing tissues. The ice is more frequently in the form of a continuous ring, or really a cylinder, extending entirely around the twig, prying apart the cells of the cortex in which it lies. The outer cylinder of cortex in such twigs is completely separated from the inner layers when frozen. In a few species instead of the continuous layer, lens-shaped ice masses are interpolated irregularly throughout the cortex. The cortical cells after the withdrawal of the water are as completely collapsed as were those in the bud scales, but they also usually regain their normal condition on thawing. In the wood ice rarely forms in large quantities. It is usually confined to small masses in the vessels themselves, or, according to some authors,[11] sometimes extends in radial plates in the pith rays. In sectioning twigs, I myself have never seen ice in the wood elsewhere than in the vessels or wood-cells. In the pith the ice, so far as I have been able to observe, always occurs within the cells and therefore in very small masses.

At the time when the buds were sectioned, cross-sections of the twigs were made and mounted in the same manner. Ice was found in the cortex of all those in which it was present in the bud, but usually in proportionately larger quantities. It was also found in the following species which showed no ice in the buds: *Corylus rostrata*, a small amount in large clefts in the cortex; *Castanea dentata*, some ice in ordinary small spaces of the cortex but not aggregated; *Hamamelis virginiana*, a ring of ice completely around the stem in young twigs. In Fraxinus, Fagus, and Juglans none could be found, and Quercus was not investigated.

Since water on freezing increases in volume, one would at first thought expect the frozen twigs to be larger in diameter than normal. Such, however, is not the case. In every instance a distinct contraction occurred, which in some cases was very marked.[12]

[11] MÜLLER-THURGAU found ice present in the large vessels of Syringa, Cornus, and in pears, almost completely filling them; and several times he could also demonstrate it in the wood-cells. The ice was the most distinct, however, in the vessels of the grape. Ueber das Gefrieren und Erfrieren der Pflanzen. II. Landw. Jahrb. 15:481. 1886.

[12] Both SACHS and MÜLLER-THURGAU have shown that a similar contraction occurs quite generally when herbaceous tissues freeze. SACHS, J., Krystallbildung

To determine the exact amount of contraction the Zeiss cover-glass measurer was used. Pieces about 10^{cm} long of one or two year old twigs taken at $-18°$ C. were inserted in the clamps of the machine, a record taken, and then the whole carried to the warm laboratory. The increase in size on thawing could be followed by watching the movement of the indicator as the ice melted, and when at last stationary another reading was taken. Some results are given in the following table:

	Frozen	Thawed	Difference	Exp. or contr.
Cornus stolonifera	2.58mm	2.60mm	0.02mm	expanded
" "	3.38	3.43	0.05	"
Tilia platyphyllos	2.03	2.10	0.07	"
" "	3.18	3.30	0.12	"
Populus dilatata	3.17	3.28	0.11	"
" "	4.72	4.80	0.08	"
Acer platanoides	2.82	2.86	0.04	"
" "	3.44	3.48	0.04	"
Pyrus Malus	2.97	3.03	0.06	"
" "	3.89	3.95	0.06	"
Salix cordata	5.34	5.44	0.10	"
" "	5.80	5.94	0.14	"

Many twigs at $-18°$ C. or below appear very much wrinkled on the surface as though dried and dead. This is especially true of the polished shoots of *Salix cordata*. On very cold mornings shoots of this species appear as though dead and dry, the bark being completely covered with fine longitudinal wrinkles. Some of these shoots were brought to the laboratory and allowed to warm, during which process the disappearance of the wrinkles could be watched with ease. In about ten minutes the twigs were entirely smooth and normal. It was from such twigs that the above readings were taken. To show more graphically the expansion during thawing, some twig-sections about 10^{cm} long were taken from the same willow and the ends while still frozen dipped in melted paraffin. The caps thus produced at the ends of the twig were in every case ruptured down the side on thawing, leaving in most cases a cleft of considerable size between the two edges. Twigs of plum were

bei dem Gefrieren u. Veränderung der Zellhäute bei dem Aufthauen saftiger Pflanzentheile. Bericht Verhand. König. Sächs. Gesell. Wiss. Leipzig, Math.-Phys. Klasse **12**: 1–50. 1860. MÜLLER-THURGAU, H., Ueber das Gefrieren und Erfrieren der Pflanzen. Landw. Jahrb. **9**: 187. 1880.

also much wrinkled, those of Negundo and apple showed a slight furrowing, those of black cherry and pear scarcely any at all.

The photographs of wrinkled twigs of *Salix cordata*, reproduced in the accompanying illustrations (*fig. 5*), were made in the open at a temperature of −20° C. The same twigs were then placed in the laboratory, and after about one hour were photographed again. The slightly wrinkled appearance in the upper shoot in

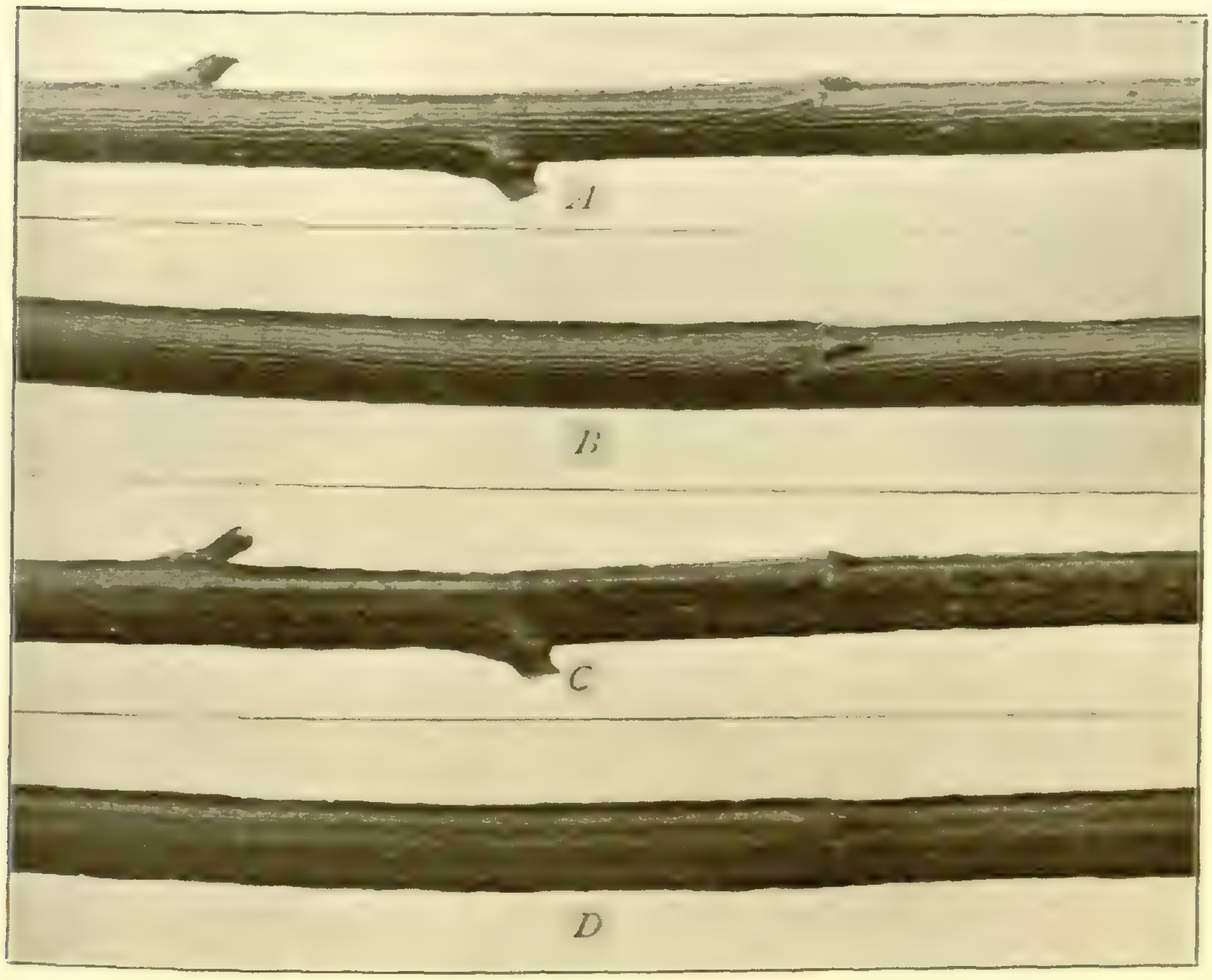

FIG. 5.—*Salix cordata:* *A* and *B*, twigs photographed in the open at −20° C., showing wrinkled condition due to contraction; *C* and *D*, the same twigs thawed in the laboratory; the furrows have disappeared except the minute normal striae on the lower twig.

the second photograph was normal for that shoot when thawed, during both winter and summer.

It seemed desirable to determine whether this contraction was mainly in the bark or in the wood or in both. At a temperature of −18° C. much wrinkled twigs of *Salix cordata* were collected, and the following measurements made:

With bark on twig the diameter, 7.80^{mm} expanded to 8.04^{mm} on thawing; difference 0.24^{mm}. With bark removed from a small spot for the clamps of the measuring instrument, the diameter, 5.05^{mm}, expanded to 5.15^{mm}; difference, 10^{mm}.

Therefore more than half of the total expansion was in the bark. Thickness of the bark was 0.5^{mm} on each side; thickness of the wood and pith, 2.05^{mm} on each side; expansion of the bark, therefore, was 13.5 per cent.; of the wood, only 2.5 per cent.

With thicker twigs, containing more hard wood, the expansion would have been still less.

Where the bark was whittled away entirely around the end of the twig and for some distance back, the expansion of the wood was not detected; probably because water had passed to the bark to freeze and being removed there was none to cause swelling again when the twig thawed.

The explanation of the contraction of twigs on freezing probably lies in the following considerations. When the water is extracted from the walls of the wood-cells, the latter contract to a slight extent just as they do when wood seasons. This accounts for a part of the shrinkage. The rest and greater part occurs in the cortex. Here the intercellular spaces are quite large and numerous, and are normally filled with air. When freezing occurs the ice forms in the spaces and the cells collapse, while the air is mostly driven completely out of the twig. The contraction in the cortex will be approximately equal to the volume of air expelled plus that of the air compressed minus the expansion of the ice while freezing. This is for contraction in all directions; only a portion of this will be radial, depending upon the structure of the particular species; much the greater part, however, is radial in all twigs.

With buds the study is not quite so easy. The record of buds measured at −18° C. and then again after thawing is shown in the adjoining table.

From this table it will seem that in all cases, except in Populus and Acer, there was a decided increase in size on freezing and a consequent decrease when thawing out. In the two named cases there was a slight contraction as in the twigs. It is not quite clear why the buds should behave so differently from the twigs. The only explanation I can offer at present is that the contraction of

	Frozen	Thawed	Difference	Contraction or expansion
Cornus stolonifera	2.22mm	2.20mm	0.02mm	contraction
" "	2.66	2.61	0.05	"
Tilia platyphyllos	3.63	3.54	0.09	"
" "	3.15	3.07	0.08	"
Populus dilatata	2.77	2.77	0.00	0
" "	3.03	3.04	0.01	expansion
Acer platanoides	5.06	5.08	0.02	"
" "	4.34	4.34	0.00	"
" "	4.16	4.17	0.01	"
Pyrus Malus	4.92	4.88	0.04	contraction
" "	4.04	4.03	0.01	"
" "	4.72	4.68	0.04	"
Prunus persica	2.62	2.59	0.03	"
" "	2.50	2.46	0.04	"
" "	2.72	2.66	0.06	"
Prunus americana	2.17	2.09	0.08	"
" "	1.78	1.71	0.07	"
" "	2.10	2.06	0.04	"

the wood is eliminated, of course, and that the formation of ice tends to bow out the scales so that they stand less closely together. If the bud scales curve like leaves in freezing this result is to be expected.

When the temperature rises sufficiently, the buds and twigs thaw out and regain their normal condition. In the sections under the microscope the reabsorption was so rapid as in most cases to be entirely completed when the ice itself had finished thawing. This results in an active sponging-out movement in the sections as the cells recover from their collapsed condition (*fig. 6*). On account of the rapidity it is frequently difficult to keep the point of observation in the field of the microscope. Thawing seems not to harm these tissues in the least, no matter how frequently or how abruptly it is done. I have often tried the experiment of transporting twigs abruptly from −18° C. to the warm laboratory at 21° C. and back again several times, thus alternately thawing and freezing them. No matter how many times this was repeated no injury could be detected in the buds, even when subsequently placed in the greenhouse to grow.

Buds and twigs do not thaw at 0° C. if the rise in the surrounding temperature is gradual, as it is in atmospheric changes, but at a much lower degree. The thawing like the freezing is proportional to the temperature, and is almost if not quite completed when the

freezing point of the tissue is reached. This, in the case of buds, lies at about $-3.5°$ C. to $-2.3°$ C. Hence, if tissues which have been subjected to $-18°$ temperature in the open are to be observed with the maximum ice content, it must be while the temperature is still low. If in the morning the temperature has risen to $-7°$ C. before observations are made, very little more ice will be found than if the cooling to $-7°$ C. had just taken place.[13]

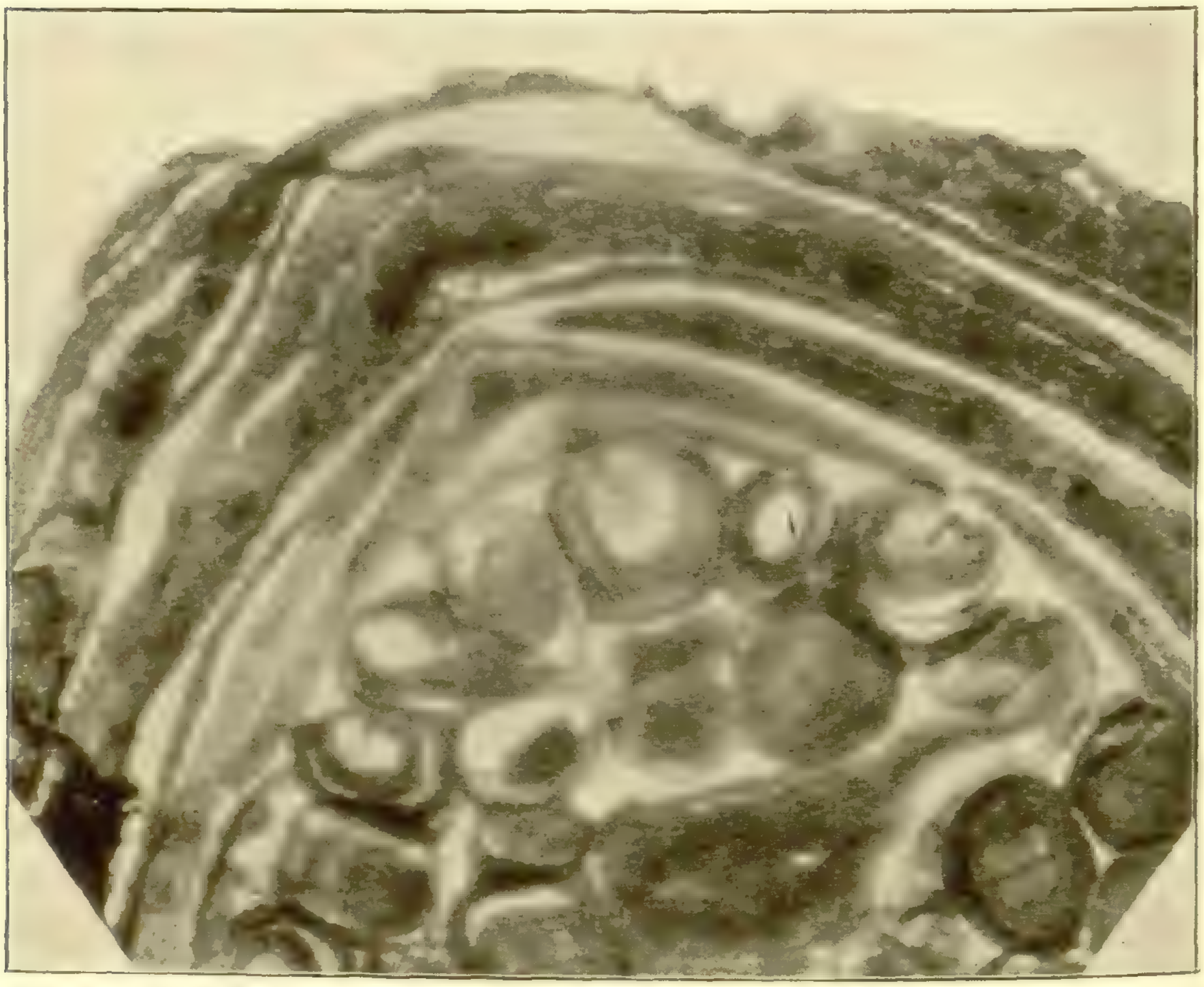

FIG. 6.—*Syringa vulgaris:* same section as in *fig. 3* thawed in the laboratory; note sponging out of tissue and closing of spaces occupied by the ice.

[13] GOEPPERT gives a similar experiment. "Twigs with buds of *Cornus mascula*, *Prunus Cerasus*, and *Aesculus Hippocastanum* were on January 2, 1871, placed ten hours at a temperature of -16 to $-20°$ C. Then while frozen stiff they were plunged into the warm tube of an oven at 25° C. and placed in water for further observation. They developed later just as others that had not been subjected to this experiment." Some other experiments with herbaceous plants led GOEPPERT to believe that in most cases alternate thawing and freezing, when taking place many times, gradually weakened the tissue. Ueber das Gefrieren, Erfrieren der Pflanzen und Schutzmittel dagegen. Stuttgart, 1883, p. 33.

WHITTEN believes that rapid thawing and freezing is very detrimental to the

WINTER FUNCTION OF BUD SCALES.

Bud scales are obviously for the purpose of protecting the tender inner shoot from detrimental external influences; but how is this protection accomplished? This is a subject regarding which opinion has varied widely and does still at the present time. I believe we shall find that the most widely accepted views, strangely enough, are not the correct ones, even though the subject appears so simple. We can conceive of such protection taking place along four lines: (1) by keeping out external moisture; (2) by preventing the penetration of cold or sudden changes of temperature; (3) by preventing the escape of internal moisture; (4) by warding off external mechanical injury. It seems best to discuss each of these in turn, and in this way determine the extent to which each one is operative.

External moisture.

There is a widespread belief that bud-scales function by keeping out the wet. The subject, however, is a difficult one to determine experimentally, and I can find no reference in literature to such experiments having been performed. Let us first determine the possible ways in which water might be supposed to injure the embryonic tissues. First, the cells might absorb too much water and thus become more sensitive to frost. It seems quite reasonable to believe that a cold spell following such an event might end the life of the bud completely. Again, through gradual absorption of the air by the water the latter might replace the air in the intercellular spaces, thus preventng free respiration. Or again, if a thawing bud were surrounded by water, the latter, instead of air, would be drawn in to fill the vacant intercellular spaces, the final result being the same as in the last case. Lastly, one might expect that the freezing of free water between the embryonic foliar and floral parts might cause

mechanical injury.

purple buds and twigs of peach. Green twigs and especially whitened ones warm up less each day and this color would therefore be protective. I believe it may quite likely be true that delicate buds might suffer by such violent treatment either from stimulated activity or increased transpiration, even though hardy trees are apparently indifferent. Das Verhältniss der Farbe zur Tötung von Pfirsichknospen durch Winterfrost. Inaug. Diss. Halle. 1902.

Taking up these in turn, if the cells were so unprotected as to be capable of absorbing water in this way, they would be expected to lose a large part again when dry conditions returned, and thus quickly following frosts alone could do harm. There is also considerable doubt whether sufficient water would be absorbed by the cells to cause any perceptible difference in sensitiveness. Water at winter temperatures absorbs air very little, and especially after having fallen in the form of raindrops it may be considered as nearly saturated. The air in the leaves would probably be absorbed very little, if at all, although compression of the air due to capillarity might allow some water to enter. If the thawing tissue has its spaces filled with water instead of air, this will not necessarily cause harm. In experiments on leaves it was found that only the ivy leaf was unable to recover when the spaces were filled with water. Many leaves allow the water to evaporate and then become normal. Mechanical injury is not probable since the air spaces of the tissue would be elastic enough to overcome the compression of the expanding ice between the organs, and after the tissue froze slight pressure from the outside on the compressed cells would do no more harm than the pressure of the ice masses within ordinary tissue.

However, the greatest objection to this theory, it seems to me, lies in the fact that protection against moisture might be obtained in a much simpler manner. The embryonic tissue might be densely clothed with strigose hairs, or densely glaucous, either of which would cause the rain drops to roll off without wetting, at the same time allowing gas-interchange to continue; or a coating of resin would effectually prevent all danger of water absorption. All of these devices are more simple than the elaborate system of bud-scales found on many trees. On the other hand, the wool produced on many buds would tend directly to retard the drying of the bud surface.

The result of an experiment may here be given. During the winter of 1902, about January 24, several buds of *Acer platanoides*, deprived of their scales but still remaining on the tree, were each inserted in a rubber pipette-bulb previously filled with water. The neck of the bulb was then fastened firmly around the twig by means of twine. The experiment was allowed to continue about one

week, during which time temperatures of −23.5° C. had alternated with those of 4.3° C., so that the buds were alternately frozen and thawed. After removal of the rubber, the tissue appeared as fresh and sound as ever; the twigs were then cut and placed with their ends in water in the greenhouse, where the treated bud remained fresh as long as did others whose scales were freshly removed as check experiments.[14]

There exists, it seems to me, insufficient evidence to sustain the theory that the exclusion of external moisture has played an important part in the evolution of scaly buds.

Heat conduction.

The popular belief is widespread that bud-scales serve to keep out the cold, and indeed such an explanation appears in some of our leading textbooks and in various other works. A moment's consideration will convince us that this cannot be true. No plant tissue yet known is a perfect non-conductor of heat, or, indeed, less than a fairly poor conductor, and scale tissue is no exception; while the very thin nature of the scaly covering on some buds, as those of Salix, would absolutely preclude their offering more than a moderate amount of resistance to the escape of heat. To keep out the cold during an entire cold spell in winter would require, even in much thicker tissue, an almost absolute non-conductivity, and that is possessed by few if any substances in nature, much less by the bud-scales. This erroneous impression has arisen probably through comparing the action of bud-scales with that of clothing upon the human body, forgetting the fact that in the body there is a constant source of heat without which clothing could not keep it warm for more than a few minutes.[15]

[14] KNY found that with the bud-scales and cortex intact average twigs will not take up as much water through these organs as they give out in dry air during a similar time. He neglected, however, to experiment with naked buds. Ueber die Aufnahme tropfbar-flüssigen Wassers durch winterlichentlaubte Zweige von Holzgewächsen. Ber. Deutsch. Bot. Gesell. **13**:361. 1895.

[15] It may be suggested that such a constant source of heat does actually exist in a tree, at least so far as the buds are concerned, and that this is provided by the heat accompanying respiration. However, reference to any textbook in plant physiology will show that the amount of heat evolved in this way is but slight in the very best examples, which are all herbs, and is mainly evident during the period of most

Such substances can only retard, not prevent, the escape of heat. As a final argument we may return to the fact that observation shows that buds are always filled with ice during cold periods, which of course could not occur if they were kept warm.

It is a more difficult matter to demonstrate whether the non-conductivity of the bud-scales is of importance to the bud in any other way. Recently GRÜSS[16] has quite firmly upheld the theory that one of their chief functions is to modify the temperatures reaching the interior of the bud. We can conceive of several ways in which such protective service might occur. First, poor conductivity might prevent injury from too rapid thawing. Second, bud-scales might prevent extreme fall of temperature by preventing excessive radiation. Third, they might prevent too frequent rapid thawing and freezing due to fluctuating sunlight, and thus prevent excessive water evaporation.

Before answering any of these questions let us try to understand a little more fully the actual relation of bud-scales to heat. This problem resolves itself into two parts, namely, a consideration of the conductivity simply, and a consideration of the relation to normal atmospheric heat changes in the open.

On the question of conductivity the following experiments seem to throw some light: Two thermometers, previously tested as to their readings, were selected, and the bulb of one was covered with the imbricated scales of a fresh horsechestnut bud, as in the previous experiment to determine the effect of color, thus forming an artificial bud with the thermometer bulb in place of the young shoot. The other bulb was left naked. The experiments were all conducted within the building where the conditions were more constant and presented fewer uncontrollable factors than outside. The room

rapid growth. During the dormant winter period it must be very slight in all trees. An ordinary thermometer probably could not measure it. It may also be suggested that since the large size and mass of the trunk would retard heat changes, being warmer than the air when the temperature is falling, and cooler when the latter is rising, this, by conduction along the branches, would modify the temperatures in the shoots and buds. SQUIRES has shown (Minn. Bot. Stud. 1:453) that the average temperature in a box elder tree was in January 1.3°C. higher than the air, in February the same as the air, and in March 1° lower. The differences between internal and external temperatures during the day was in all cases only a few degrees. The idea that the branches can conduct such slight modifications so long a distance without loss is so evidently unreasonable as to require no more discussion here.

[16] GRÜSS, J., Beiträge zur Biologie der Knospen. Jahrb. Wiss. Bot. **23**:651. 1892.

selected had a temperature ranging from 3.7° C. to 4.3° C. during the several days on which readings were taken. The two thermometers were brought to the same reading in a warmer place, either in another room or over a water bath, then quickly taken out and the readings recorded for every few seconds until they again registered at the same degree in the cold atmosphere of the room. Two classes of readings were taken, one from a temperature only a few degrees above that of the cold room and the other from one far higher. The readings in each class, taken with the same bud, corresponded remarkably. A specimen reading from each set is here given.

TABLE II.

Horsechestnut bulb and naked bulb transferred abruptly from a temperature of 19.5° C. to one of 3.5° C. (See *fig.* 7.)

Naked bulb	Horsechestnut bulb	Time	°Difference	Time difference
67° F.	67° F.	0 sec.	0 F. (0° C.)	0 sec.
66	67	5	1 (5.0)	20
65	67	10	2 (1.0)	20
64	67	18	3 (1.5)	37
63	66	25	3 (1.5)	45 (¾ min.)
62	65	30	3 (1.5)	50
61	65	38	4 (2.1)	72
60	65	45	5 (2.7)	60
59	64	55	5 (2.7)	75
58	63.5	60	5.5 (3.0)	95
57	63	70	6 (3.3)	100 (1⅝ min.)
56	62	80	6 (3.3)	105
55	62	90	7 (3.9)	125
54	62	100	9 (5.0)	145
52	61	110	9 (5.0)	175
50	59	130	9 (5.0)	205
49	58	155	9 (5.0)	200 (3⅓ min.)
47	56	185	9 (5.0)	255
46	56	200	10 (5.5)	285
45	55	215	10 (5.5)	340
44	54	245	10 (5.5)	375
43	52	285	9 (5.0)	420 (7 min.)
42	51	325	9 (5.0)	520
41	49	355	8 (4.4)	630
40	48	395	8 (4.4)	765 (12½ min.)
39	47	440	7 (3.9)	765
39	46	485	6 (3.3)	765
39	45	555	6 (3.3)	960
39	44	620	5 (2.7)	960
38	43	705	4 (2.1)	1235 (20½ min.)
38	42	845	3 (1.5)	
38	40	1160	2 (1.0)	
38	39	1515	1 (0.5)	
38	38	1940	0 (0.0)	

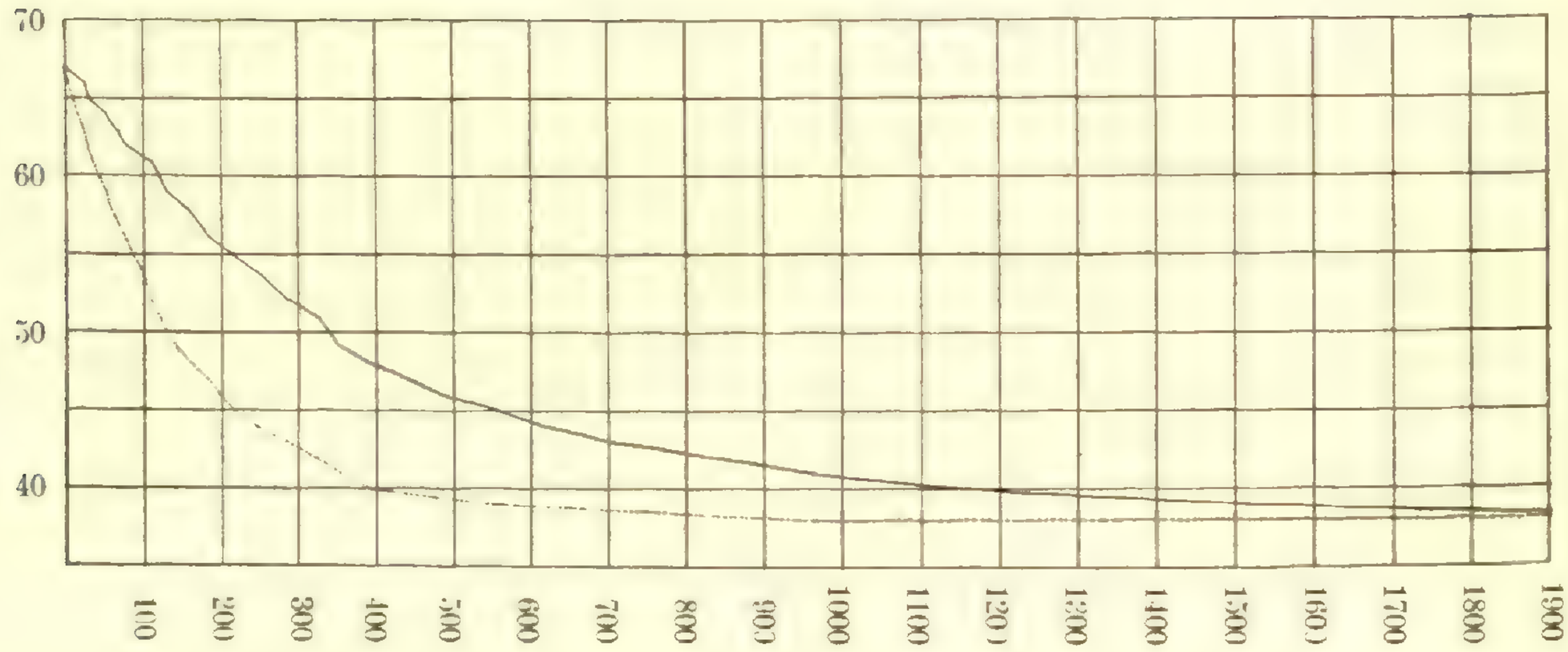

FIG. 7. ——— horsechestnut bulb; ..... naked bulb. Abscissas represent 5° F.; ordinates, 100 seconds. See Table II.

The first column of figures represents the readings in degrees from the thermometer with the naked bulb; the second column the same from the bud-covered instrument; the third column shows the time in seconds from the beginning of the experiment; the fourth column the difference in degrees at each reading; and the fifth column is the "time-difference," so-called, which represents the number of seconds elapsing after a reading on the naked bulb before the same temperature was reached on the horsechestnut bulb, in other words, the number of seconds by which the bud-scales retarded the fall of temperature in the enclosed bulb.

While not attempting to deduce the physical laws governing the fall of temperature in each case, we may note from the tables and curves several points which bear upon our problem. It will be seen that theoretically the time required for the temperature to fall in either case is infinitely long, the curve becoming nearly horizontal towards the end of each experiment. But for all practical purposes, and as closely as my instruments would measure, the fall was completed in about thirty minutes in each case. The greater part of it, in fact, was completed in ten minutes. As regards time, in Table II the very much more rapid radiation of heat more than balanced the effect of the greater quantity of heat to be radiated.

As we should expect, the retarding effect of the bud-scales in degrees, shown in the fourth column, was much greater in case of the greater extremes of temperature, and was greatest when the

TABLE III.

The same thermometers and bud preparation transferred abruptly from a temperature of 51° C. (over a water bath) to one of 2.7° C. (See *fig. 8.*)

Naked bulb	Horsechestnut bulb	Time	°Difference	Time difference
124° F.	124° F.	0 sec.	0° F. (0° C)	0 sec.
120	123	5	3 (1.5)	15
116	122	10	6 (3.3)	15
110	120	15	10 (5.5)	27
106	118	20	12 (6.6)	28
100	116	25	16 (8.8)	42
97	114	30	17 (9.4)	45 ($\frac{3}{4}$ min.)
94	113	35	19 (10.5)	50
91	111	40	20 (11.1)	55
88	109	45	21 (11.6)	60
84	106	48	22 (12.2)	77.7
82	105	55	23 (12.7)	80
80	103	65	23 (12.7)	80
77	101	65	24 (13.3)	90 ($1\frac{1}{2}$ min.)
74	99	69	25 (13.9)	111
72	97	75	25 (13.9)	115
70	96	80	26 (13.9)	120
69	94	85	25 (14.4)	125
67	93	90	26 (13.9)	135
65	91	95	26 (14.4)	145
63	88	105	25 (13.9)	155
61	87	115	26 (14.4)	160
60	86	120	26 (14.4)	165
58	83	130	25 (13.9)	180
58	81	140	23 (12.7)	180
54	76	160	22 (12.2)	200 ($3\frac{1}{3}$ min.)
52	75	170	23 (12.7)	230
50	74	180	24 (13.3)	250
49	72	190	23 (12.7)	265
47	69	210	22 (12.2)	280
46	68	220	22 (12.2)	330
45	65	240	20 (11.1)	335
44	63	260	19 (10.5)	240
42	61	275	19 (10.5)	435 ($7\frac{1}{3}$ min.)
41	59	305	18 (10.0)	465
41	57	320	16 (8.8)	465
41	56	335	15 (8.3)	465
40	55	355	15 (8.3)	545 (9 min.)
40	53	380	13 (7.2)	545
39	51	420	12 (6.6)	580
39	49	455	10 (5.5)	580
38	47	490	9 (5.0)	990 ($16\frac{1}{2}$ min.)
38	46	550	8 (4.4)	990
37	44	600	7 (3.9)	1300 ($21\frac{1}{2}$ min.)
37	43	660	6 (3.3)	
37	42	710	5 (2.7)	
37	41	770	4 (2.1)	
37	40	900	3 (1.5)	
37	39	1000	2 (1.0)	
37	38	1480	1 (.5)	
37	37	1900	0 (.0)	

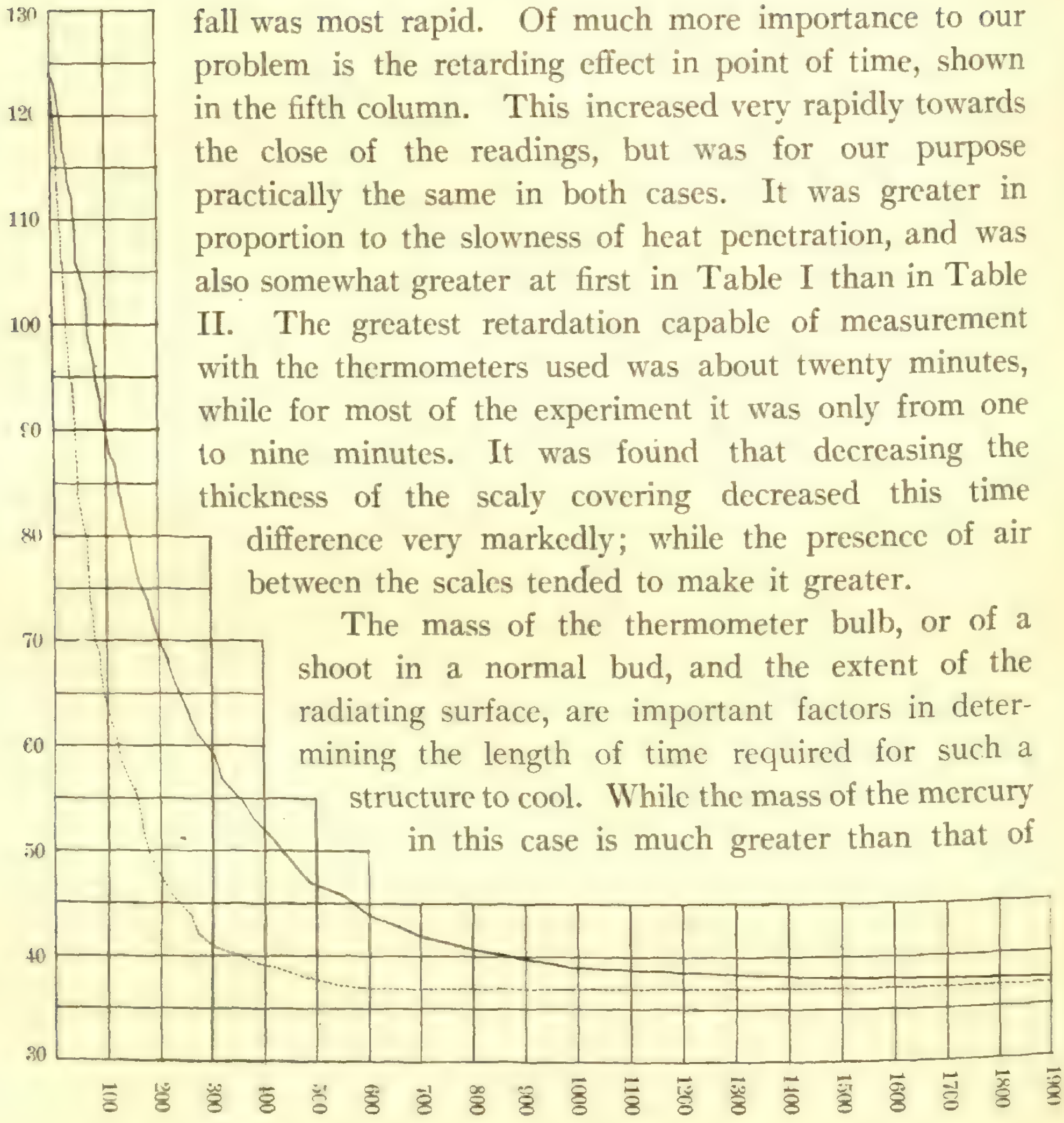

FIG. 8. ——— horsechestnut bulb; ..... naked bulb. Abscissas represent 5° F.; ordinates, 100 seconds. See Table III.

fall was most rapid. Of much more importance to our problem is the retarding effect in point of time, shown in the fifth column. This increased very rapidly towards the close of the readings, but was for our purpose practically the same in both cases. It was greater in proportion to the slowness of heat penetration, and was also somewhat greater at first in Table I than in Table II. The greatest retardation capable of measurement with the thermometers used was about twenty minutes, while for most of the experiment it was only from one to nine minutes. It was found that decreasing the thickness of the scaly covering decreased this time difference very markedly; while the presence of air between the scales tended to make it greater.

The mass of the thermometer bulb, or of a shoot in a normal bud, and the extent of the radiating surface, are important factors in determining the length of time required for such a structure to cool. While the mass of the mercury in this case is much greater than that of the shoot, its specific heat being only one-thirtieth that of water would render the two not very dissimilar, so far as the present problem is concerned. In apparent volume they do not differ greatly, so that the radiation surfaces of the two would be nearly the same.

I believe we are justified in saying that a normal horsechestnut bud would not behave in any essential way differently from the artificial one here used; and that the time for it to cool off would

be for all practical purposes not over about thirty minutes, no matter whether it was cooled very much or only a little, providing it was plunged directly into the cooler temperature.

We may also say, I believe, that smaller buds with thinner scales and smaller shoots will show a time period correspondingly less than thirty minutes, and a time difference which will approach more nearly zero. In the case of the willow buds with only one thin bud-scale, the time period and time difference must be very small indeed.

A number of readings were taken in which the thermometers were warmed up instead of cooled, and it was found, as expected, that the above generalizations applied in this case also. Providing that atmospheric changes out of doors are abrupt, I fail to see how the temperature at the center of a bud of medium size can be retarded more than five or ten minutes over practically all of the range of fall. A small bud would probably be retarded only about one to five minutes. Of course the retarding would be greater than this through the last degree and fraction of a degree, but this slight change, it seems to me, would be of little moment to the present question.

Buds in nature, however, are under slightly different conditions. Instead of being transported from one temperature to another, the temperature itself changes. We should therefore conduct some experiments in which the air itself is varied. This change is either very gradual, as when a thaw approaches, or more abrupt, as when the sun shines from behind a cloud upon the bulb, which is the only way in which abrupt changes are produced in nature. In either case they are much less violent than were our laboratory experiments. During warming by the sun, radiation from surrounding objects may play an important part and introduce still another factor. We should therefore conduct some experiments in which the air itself is warmed. The experiments with the horsechestnut bud already described in the discussion of the function of color are to the point here. They show in a surprising way that instead of retarding the rise in temperature within the bud, under these very natural conditions the bud-scales actually seem to hasten it.

These experiments were with direct sunlight. It seemed possible

that the readings might be different if radiated instead of direct heat was employed, especially since there is a considerable difference in the nature of such heat, as shown by the well-known fact that direct heat from the sun passes easily through glass into the greenhouse, but when radiated passes out with much greater difficulty, thereby warming the house.

A number of readings taken with naked and horsechestnut bulbs transferred from the shade to the surface of a black book in direct sunlight with the bulb raised 3–4^{mm}, or with the bulbs projecting several inches over the edge of the book which itself was raised several feet from the ground, or with the bulbs raised 7.5–10^{cm} above the surface of the book, showed no appreciable difference that could be referred to a difference in kind of radiated heat. There was some difference in the readings, of course, but this could be traced directly to the fact that there was more intense heat where the heat of radiation was also present. In case of more intense heat the extra absorbing power of the bud-scales was at first more obscured by the slightly greater retardation of heat-penetration due to the greater difference in outside and inside temperatures, as we should expect from the deductions from Tables III and IV. This was partially shown by the difference in locality of the crossing of the two curves plotted from each reading.

Looking at the matter from still another standpoint, we may consider how much time is required for a bud to thaw. As shown by the cover glass measurer, the wrinkled willow twigs thawed and became perfectly normal in thirty minutes at the temperature of the laboratory. Undoubtedly the ice had disappeared in about half the time. Large buds of horsechestnut will lose all their ice in about twenty-five minutes under similar conditions, and buds of Negundo in about fifteen minutes. The small buds of the black cherry require only about ten minutes for thawing. The time required in the laboratory for the various buds, therefore, is ten to thirty minutes. The question is whether when the temperature changes are slow the buds thaw proportionately more slowly. The answer must be that they will, slightly, just as a cake of ice will thaw more slowly when the temperature rises gradually than when the rise is abrupt. This difference is proportional to the size of the

ice cake, since it depends largely upon the non-conductivity of the ice and the greater quantity of heat required to convert ice into water. This heat is more slowly available when the change is gradual. Although no experiments were made under these conditions, it is to be expected, I think, that with long slow rise in atmospheric temperature, the retarding effect would almost if not quite disappear. Frozen peach buds, placed in the air at −5.5° C., which gradually rose in 2 to 2.5 hours to a temperature of −1.0° C., were completely thawed, apparently as soon at the temperature reached about −2.3° C., thus following the general rule for frozen tissue.

We are now in position to consider the questions outlined on a previous page regarding the various ways in which the bud-scales may be supposed to act beneficially by modifying the temperature.

It was first suggested that they might retard the thawing out and thereby be of benefit to the bud. From the tables already given and the observations regarding them, it becomes at once apparent that the temperature modification which scales are capable of producing are, in the cases of moderate sized buds, of very little moment—not more than two or three minutes during most of the time, and then only when the change from one temperature to another is abrupt. When the transition is gradual, the retarding effect will be very slight indeed, and is frequently wholly offset by the absorbing power of the darker color. I cannot see how under any atmospheric condition the modifications can become great enough to be noticeable unless careful measurements are taken. The idea that a slow thawing is beneficial to plants has come about from analogy with the frosting of human tissue and from the consideration of the treatment which gardeners successfully give frosted plants. But the gardener's treatment consists in keeping the plant cool and dark for hours or even days after the freezing; while recent investigators have shown that slow or rapid thawing (*i. e.* conversion of the ice into water) in themselves bear no relation whatever to the extent of the injury. The gardener's treatment is essentially an after-treatment—while the plants are recovering from the shock. I have already cited the fact that buds of many trees, at least, may be thawed in an oven and then frozen alternately many times and still come out in the greenhouse apparently

as fresh as others not so treated.[17] The answer to this first question then, is, that bud-scales do not function by preventing rapid thawing of winter buds; neither does bark so function towards the twigs.

It has been suggested that bud-scales protect the bud by preventing rapid radiation from the delicate tissue during the cold nights, and thereby preventing a harmfully low fall of temperature. MÜLLER-THURGAU,[18] by placing one thermometer on some cotton under a 0.5^{sqm} cloth screen fastened 1^{dm} above the ground, and another thermometer outside, was able on a clear night to get 4° C. difference due to radiation. GRÜSS[19] states that differences in temperature due to radiation may be one or two degrees on cool nights just before sunrise, and as great a difference as 6° C. has been observed by other investigators. A difference of 4–6° C. would frequently be of importance to tender exotic buds in winter, but it is scarcely to be supposed that so slight a difference would be of much moment to the great majority of perfectly hardy species which withstand all of the fluctuations of our vigorous American climate without injury. Indeed these species seem capable of existing below any atmospheric temperature that has yet occurred in this country, as freezing mixture experiments have shown. Besides, the structure of buds does not lead one to expect a radiation screen as efficient as those specially constructed. Strictly speaking, the question here is not one of radiation of heat, since the scales are all more or less in contact, but of conduction, and as such has already been treated.

HENSLOW[20] has shown that it seems desirable for plants in temperate regions to protect their delicate bud-structures from loss of water when the bud is opening. Such loss he says is favored by radiation and heat absorption. The above objection will apply here also for the first part of this last statement, and the latter part is treated elsewhere in this paper.

[17] MOLISCH, H., Untersuchungen über das Erfrieren der Pflanzen. Jena. 1897

[18] MÜLLER-THURGAU, H., Ueber das Gefrieren und Erfrieren der Pflanzen. Landw. Jahrb. **15**:563. 1886.

[19] GRÜSS, Beiträge zur Biologie der Knospe. Pringsh. Jahrb. **23**:651. 1891–92.

[20] HENSLOW, G., On vernation and the method of development of foliage as protective against radiation. Jour. Linn. Soc. Bot. **21**:624. 1886.

In December 1901 some experiments were conducted to show whether twigs and buds while continuing frozen lost as much water by evaporation as when alternately thawed and frozen several times during the same period. It was found that they did not quite, and hence the question whether bud-scales may function by preventing too frequent thawing and freezing. Several buds of *Pinus Laricio* and horsechestnut, also several twigs 15^{cm} long of *Syringa vulgaris* and apple, were sealed at the cut end with Venice turpentine, weighed, and quickly placed on a tray in the open air. They were divided into two equal lots, and one of these was brought into the warmer laboratory for a few moments ten times, thus insuring ten alternate thawings and freezings. During the experiment, which lasted three days, the temperature ranged from −18° C. to −7° C. in the open. The results were as follows:

Horsechestnut buds continued frozen lost 0.4% of their water.
" " alternately thawed and frozen lost 0.6% of their water.
Pinus Laricio buds continued frozen lost 3.4% of their water.
" " " alternately thawed and frozen lost 5.0% of their water.
Syringa twigs continued frozen lost 1.3% of their water.
" " alternately thawed and frozen lost 2.4% of their water.
Apple twigs continued frozen lost 1.6% of their water.
" " alternately thawed and frozen lost 2.4% of their water.

In every case there was a greater loss of water from the buds which were alternately thawed and frozen. The difference was very marked, and in each case amounted to about one-third of the total loss. Considering the total quantity of water present, this was really a very slight increase in loss, however, being 0.25% in horsechestnut, 1.1% in lilac twigs, 0.8% in apple twigs, and 1.2% in pine buds; and with me it is a serious question whether, in all of these cases, so slight a difference would not be quickly equalized during spells of thawing by conduction from the older wood, if the twigs and buds were connected with the trunk in the normal manner. Again, the thawings in nature would probably be fewer, and it has not been shown that bud-scales prevent such thawings. It seems to me that here again a beneficial functioning of the bud-scales is very doubtful.

But the most vital argument against all these cases lies in the fact that experiments have shown that dark buds tend actually to

increase the heat absorption. Therefore, these considerations could scarcely have been instrumental in bringing about the existence of such structures.

The idea that bud-scales may protect the bud by warding off the hot rays of the sun applies mainly to the tropics. It seems to have been first advanced by TREUB,[21] who cites several cases, where in plants exposed to the hot tropical sun delicate young tissues were inclosed in enlarged stipular organs or else well-shaded by overlapping leaves or by other special structural provisions.

On the same subject, in 1891 another paper was published by POTTER.[22] According to this investigator many trees in the tropics protect their young leaves and shoots from direct sunlight by means of stipules. These organs were removed from a number of buds and in every case the leaves from these when mature were deformed and abnormal. The sunlight seemed to produce injury by causing more water to be evaporated than could be replaced. For this reason Artocarpus, the most pronounced type of this class, unlike most trees, produced leaves throughout the dry season, probably because of the stipular protection. Instead of by stipules some tropical plants obtain similar protection by various methods of leaf-folding, shading by older leaves, and coating with gum. Is there not inaccuracy here in his interpretation? Rather than by actually preventing the entrance of heat from the sun, which it seems such structures could do only to a slight extent, is it not more probable that they function simply by preventing the escape of extra moisture vaporized by the intense heat?

The relation of bud-scales to the young shoot when the bud is opening is discussed under internal moisture relations. Suffice it to say that the results reached seem to indicate that even in this case the scales do not function beneficially by modifying the heat.

It has sometimes been thought that the layers of hair and wool found in many buds, as for example in the horsechestnut, are for the purpose of modifying the heat conditions inside. To obtain

[21] TREUB, M., Iets over knopbedekking in de tropen. Hand. van. het eerste Nederlandsch Natuur- en Geneeskundig Congres. Amsterdam. 1887, p. 130. Ref. Bot. Centralb. **35**:328. 1888.

[22] POTTER, M. C., Observations on the protection of buds in the tropics. Jour. Linn. Soc. Bot. **28**:343. 1891.

evidence upon this point I performed the following experiment. The two thermometers used in the previous experiments were selected, and the bulb of one was coated with black cloth; that of the other was wrapped in a layer of cotton about twice the thickness of the wool in the horsechestnut bud, and was then coated with black cloth. The surface of both bulbs was therefore black.

TABLE IV.

Bulb covered with black cloth, and bulb covered with cotton and black cloth; transferred from a temperature of 56° C. to a room of 9° C.

Cloth bulb	Cotton bulb	Time	° Difference	Time difference
134° F.	134° F.	0 sec.	0° F. (0° C.)	0 sec.
130	131	5	1 (0.5)	7
128	131	10	3 (1.5)	10
125	128	15	3 (1.5)	15
123	128	20	5 (2.7)	15
222	127	25	5 (2.7)	15
119	125	30	6 (3.3)	20
117	123	35	6 (3.3)	20
114	121	45	7 (3.9)	20
111	119	50	8 (4.4)	25
109	117	55	8 (4.4)	26
106	115	60	9 (5.0)	30
104	112	70	8 (4.4)	30
100	110	80	10 (5.5)	37
98	107	85	9 (5.0)	40
96	105	95	9 (5.0)	40
93	103	105	10 (5.5)	40
91	101	113	10 (5.5)	42
89	99	120	10 (5.5)	45
87	97	130	10 (5.5)	45
86	96	135	10 (5.5)	43
84	93	145	9 (5.0)	50
82	91	155	9 (5.0)	55
80	89	165	9 (5.0)	55
78	88	173	10 (5.5)	62
77	86	178	9 (5.0)	72
76	85	185	9 (5.0)	75
74	84	195	10 (5.5)	75
73	83	205	10 (5.5)	80
72	82	210	10 (5.5)	85
71	81	215	10 (5.5)	95
70	79	225	9 (5.0)	95
69	78	235	9 (5.0)	100
68	77	250	9 (5.0)	105
67	76	260	9 (5.0)	110
66	74	270	8 (4.4)	110
65	73	285	8 (4.4)	120
64	72	295	8 (4.4)	130
63	71	310	8 (4.4)	135
63	70	320	7 (3.9)	135
62	69	335	7 (3.9)	140

TABLE IV.—*Continued.*

Cloth bulb	Cotton bulb	Time	° Difference	Time difference
61°F.	68°F.	355 sec.	7°F.(3.9C°)	155 sec.
61	68	370	6 (3.3)	155
61	66	380	5 (2.7)	155
60	65	405	5 (2.7)	145
59	64	425	5 (2.7)	160
58	63	445	5 (2.7)	185
57	65.5	475	5.5 (2.7)	210
56	61	510	5 (2.7)	270
56	60	550	4 (2.1)	310
55	59	585	4 (2.1)	545
54	58	630	4 (2.1)	545
54	57	685	3 (1.5)	545
53	57	745	4 (2.1)	1083
53	56	780	3 (1.5)	1083
53	55	895	2 (1.0)	1083
52	55	1015	3 (1.5)	1300
52	54	1175	2 (1.0)	1300
51	52.5	2155	1.5 (.8)	480
49	51	2635	2 (1.0)	1440
49	50	3355	1 (.5)	
48	49	4075	1 (.5)	

As expected, the retarding effect was apparent in this rather violent experiment, but it was not great. The maximum degree difference of 10° was less than one-half that produced by the bud-scales in Table III, while the time difference through the greater part of the experiment ranged from 0 to 4 minutes. I think it may be inferred that the wool in the horsechestnut bud retards the penetration of heat, when the changes are at all great, by 0.5–3 minutes. At any rate it seems evident to me that the retarding power of the wool in such buds as horsechestnut is insufficient to explain the presence of such a structure. This appears not only from experiment but from a general consideration of the thinness of such structures compared with the relatively great temperature differences which they are supposed to offer protection against, and must in order to be effective. Their true function, it seems to me, lies in an entirely different direction, as we shall see somewhat later.

In concluding this study of the relation of bud-scales to temperature the following summary may be made:

Bud-scales or bark cannot "keep out cold" during the cold spells of winter.

They seem not to modify the temperature sufficiently to be of

beneficial importance in preventing rapid changes, even if such changes are detrimental.

Rapid thawing in itself is probably not detrimental to buds and bark.

Bud-scales seem of no benefit in keeping out the heat from sudden bursts of sunshine. They do not appreciably prevent the loss of water by preventing alternate thawing and freezing. They do not retard radiation to any important degree.

Dark-colored bud-scales indeed, instead of preventing temperature changes actually seem to absorb more heat than if they were lighter colored.

"Wool" in buds does not function by modifying temperature changes.

Bud-scales do not seem to function in modifying temperature changes when the bud is opening.

Bud-scales may protect the delicate tissues in the tropics from heat, but it would seem rather from excessive transpiration due to great heat than from the heat itself.

Finally, we may conclude that as a factor in the evolution of buds and bark in cold climates temperature considerations have probably played a very minor part.

Internal moisture.

Of all the more important factors concerning the function of bud-scales, perhaps that relating to their inhibiting effect upon the loss of internal moisture is the least recognized by people in general. In scientific literature, however, it has received considerable attention. Most authors now consider this one of the principal functions of the bud-scales and also of the bark. The subject has been discussed briefly by CADURA[23] and GROOM,[24] but also more fully by GRÜSS,[25] who performed a number of experiments to demonstrate the point. His results may be summarized as follows. The first function of the scales consists in protecting the inner meristematic

[23] CADURA, R., Physiologische Anatomie der Knospendecken dicotyler Laubbäume. Breslau. pp. 42. 1887.

[24] GROOM, P., Bud protection in dicotyledons. Trans. Linn. Soc. II. 3:255. 1893.

[25] GRÜSS, J., Beiträge zur Biologie der Knospen. Jahrb. Wiss. Bot. 23:649. 1892.

tissue from loss of water. Even in summer and especially in fall, when the sap flow decreases, the tender embryonic interior of the bud must be protected from too great transpiration. Also in winter this function is not interrupted, for then the cold wind can bring into play its desiccating action. To prevent loss of water, cork layers are formed, or in place of these felty hairs may be produced. A third method consists in the excretion of resin. If, under constant temperature, the scales were removed from an oak bud, it soon died, even though there was a moderate amount of moisture present. The inner bud-scales dried out and perished, as well as the embryonic tissues. The young leaves of a beech bud so deprived of scales persisted much longer than did those of the European oak; which he thinks was because the former were hairy while the latter were not. Buds of horsechestnut proceeded to develop in spite of the removal of the scales, probably, he thinks, because of the thick wool among the young parts. Buds of *Abies pinsapo*, whose pitch had been removed by carbon bisulfid, dried out in a very short time. These experiments were all performed on twigs cut from the trees and placed in water.

In 1895 KNY[26] published a paper dealing with the transpiration and absorption of water by buds and twigs in winter. He cites WIESNER and PACHER as having shown that horsechestnut loses water from twigs in winter, and also HARTIG as having shown that many trees do the same. Experiments are given to show that in general not so much water is absorbed by these parts in saturated atmosphere as may be given off at an ordinary degree of saturation.

In 1895 some determinations of the amount of water lost by twigs with buds attached were made by the Cornell Experiment Station.[27] The experiments lasted three days, beginning April 7. The twigs were sealed at the cut end and kept in an open shed. The percentage of loss ranged from 2 to 10%, with an average of 5.4%.

In 1875 WIESNER and PACHER[28] found that twigs of horsechest-

[26] KNY, L., Ueber die Aufnahme tropfbarflüssigen Wassers durch winterlich-entlaubte Zweige von Holzgewächsen. Ber. Deutsch. Bot. Gesell. **13**:361. 1895.

[27] BAILEY, L. H., Cornell University Experiment Station Rep. **1896**:4.

[28] WIESNER u. PACHER, Ueber die Transpiration entlaubter Zweige und des Stammes der Rosskastanie. Oesterr. Bot. Zeitschr. No. 5. p. 9. 1875.

nut transpired an appreciable amount in winter at a temperature of 13–17° C. and a slight amount also at −10° C. This was true in older twigs. The leaf scars transpired more than the periderm. The winter buds also lost some water.

That there is actual loss of water in winter probably every one knows. My experiments given below show this very definitely, but perhaps few understand that there can be a loss when the tissue is frozen as well as when thawed, though less in extent. Water may evaporate to a large extent from ice crystals themselves, as is shown by the drying of frozen soil, damp clothing, and the frequent disappearance of small quantities of snow at temperature below the freezing point. In buds not all of the water becomes ice, and the remainder is free to evaporate as at a higher temperature.

The fall of temperature on the approach of winter is always accompanied by a decrease in the power of root absorption, and it has been shown that to a certain extent absorption is proportional to the temperature. In the case of our native plants, the decrease must be very considerable when the zero air temperatures have chilled the soil to a depth of many feet. A compensating decrease in transpiration must occur or otherwise the cells will suffer from too small water content. This is mainly accomplished by the fall of the leaves, but is greatly aided also by the coverings of the bud and the waterproof bark. But so far bud-scales would not be a necessity, because very little root absorption would probably be sufficient to supply the slight amount of water that could evaporate from unprotected buds, compared with that necessary to supply the leaves. Besides, it has been found that considerable water is present in the wood at all times, and in some trees even a larger amount than in the summer. The necessary factor, I suspect, lies in the decreased osmotic activity and vigor of the young tissue itself. During the summer the tendency to transpire is probably, a large share of the time, not so great as in the winter and spring because of the greater saturation of the air; but there are times during the summer when the transpiration is very great indeed. The young tissues do not then dry up very readily, so that little harm usually results. At this time I imagine the growing cells are osmotically very active and more easily draw to themselves

a supply of water sufficient to offset that lost in transpiration. In winter, however, the cells are inactive, and on account of the cold the osmotic force is much decreased, so that the cells find it impossible to resupply quickly the transpiration water when this function is very great.

That loss of water beyond a certain point is detrimental to the cell needs no further demonstration. It has been shown that each cell demands a certain percentage of water, depending mainly upon its activity and water content, in order to maintain its life-properties. If transpiration even for a short time reduces the water in the cells of the bud below the critical percentage, the cells will cease to remain alive.

During January 1901, I cut some twigs of horsechestnut, stripped off the bud-scales from some of the buds, and exposed the whole to an outside temperature of −18° C. to −12.3° C. for 24 hours, after which the twigs were placed with their cut ends in water in the greenhouse for further development. The buds all lived, although those without bud-scales were the first to commence growth. Subsequent experiments show that the reason why none died was because the exposure to the dry air was not long enough. On March 1 of the same year, buds of the black cherry, *Crataegus punctata*, horsechestnut, lilac, apple, and *Pinus Laricio* while still on the tree were deprived of their scales and each divided into two lots. One lot was left naked, the other was varnished completely with Venice turpentine to prevent loss of water. When the normal buds were opening May 8–10 it was found that both varnished and naked buds were all dead except on *Pinus Laricio*. On this plant the naked buds were all dead, but the varnished ones were alive, and later all developed into normal shoots.

The varnished buds in all cases seemed to be all sound and turgid until warm weather and time for swelling came, when they seemed to decay rapidly, and in no case except the pine did any swelling occur. I suspect that death here was due to the retardation of respiration owing to the lack of oxygen. The pine is normally closely surrounded by resin without a space inside as in horsechestnut, and possibly some other way is here provided for obtaining oxygen. The pine, therefore, is the only one of the series in which

the results of varnishing are important to us here. The naked buds of pine in every case began to dry and shrivel up after only a few days' exposure, and were quite dead long before the time for them to open. There seems no doubt whatever that the varnish preserved the pine buds by preventing loss of water. Without the varnish the pine was one of the first to succumb. That this thin layer of varnish replaced effectively the thick layer of scales is also good evidence toward the idea that the scales do not function by causing temperature modifications.

To determine just how much more water is lost from buds without scales the following experiment was performed. Several buds of *Pinus Laricio* and horsechestnut were separated from the trees by an incision at the base of the bud and the scales were removed from all. One-half were quickly varnished, weighed, and placed in the open air at −18° C., while the other half without varnish were weighed and exposed at once. Care was taken to seal up the cut end in both sets so that no water could escape that would not if the buds had remained on the trees. After three days at a temperature of −18° C. to −7° C. the results were as follows:

	Orig. weight	Final weight	Dry weight in oven	Per cent. H_2O lost in exp.
Pinus Laricio—continued frozen:				
With bud-scales	2.65gm	2.615gm	1.35gm	2.7
Without bud-scales	1.28	0.96	0.58	45.0
Aesculus hippocastanum—cont. frozen:				
With bud-scales	4.03	4.02	1.80	0.4
Without bud-scales	2.21	1.94	1.04	33.0
Same alternately thawed and frozen 10 times during experiment by bringing into the laboratory:				
With bud-scales	5.30	5.28	2.26	0.65
Without bud-scales	2.01	1.66	0.93	32.0
Syringa vulgaris—continued frozen:				
With bud-scales	1.15	1.135	0.52	2.8
Without bud-scales	0.41	0.32	0.18	30.0

I think that nothing could show better than these experiments the very great difference in amount of water transpired by buds protected by scales and those having none. No wonder that the loss of water oversteps the critical point and causes the destruction of the tissues.

The buds in the above experiment were separated from the tree upon which they grew, and therefore could not receive water from it to replace that transpired. It would be interesting to know how much water moves into the bud to replace the quantity lost, thus giving a better idea of the actual decrease in percentage within the cells. This has not been done for twigs at temperatures above freezing, but the following figures are available for the frozen buds of pine. I selected six vigorous buds of *Pinus Laricio*, all on the west side of the tree, deprived them of their bud-scales and allowed them to remain exposed three days. The temperature during this time ranged from −18° C. to −6.7° C., so that the twigs as well as the buds were constantly frozen. Three of the buds were cut off, the cut surface sealed, and placed in a tray at the base of the tree, while the other three remained attached. The results were as follows:

	Wt. at end of exp.	Dry weight	Difference	Per cent. H_2O present at end of exp.	
Cut buds—					
No. 1	0.105gm	0.061gm	0.044	41.9	41.2 average
" 2	0.150	0.090	0.060	40.0	
" 3	0.230	0.134	0.096	41.8	
Not cut—					
No. 1	0.255	0.139	0.116	45.5	47.5 average
" 2	0.240	0.121	0.119	49.5	
" 3	0.210	0.110	0.100	47.6	

It seems, therefore, that there was a rise of about 5% of water into the bud while the tissues were frozen. This is quite possible, since only a portion of the water was converted into ice, the remainder remaining fluid in the walls and protoplasm and still capable of movement. The figures given above for the loss of water from desquamated buds are therefore slightly too large in every case. It may be noticed by computing corresponding figures that the loss of water during this last experiment is slightly less than in the two previous experiments in which desquamated buds of *Pinus Laricio* were used. This was due to the fact that the last experiment was conducted in a different place, on the other side of the building. I have found that exposure makes a very considerable difference in the loss of water, and readings which are to be compared must

be taken in the same place under the same weather conditions. The rise of 5 per cent. is only a small part of the whole water lost. How much will rise into the bud when the tissues remain thawed was not determined.

The question arises whether the damage to the bud is done while the latter is frozen or thawed. I believe that injury is done both while thawed and while frozen, for the reason that the loss of water from unprotected buds is sufficiently great to cause death at either time. However, probably more damage is done above freezing point, because here the loss must increase with the temperature much faster than does the conduction of water in the bud.

The evidence therefore seems to be sufficient to warrant the conclusion that the loss of water during the winter is a danger against which the bud-scales and bark serve as protective organs. Next to the warding off of mechanical injury this is probably their most important function.

Protection of the young shoot.

In many trees, notably in maples, horsechestnuts, oaks, etc., the growth of the young shoot is accompanied by a growth in length of the bud-scales, especially of the inner ones, so that a sort of telescopic tube is formed in which the young shoot remains concealed, frequently until a growth of 2–8^{cm} has taken place. The function performed by the bud-scales at this time has long been a subject of interest to investigators, among whom GRÜSS[29] has given us the most comprehensive account.

According to GRÜSS, the protection lies in the ability of the scales to prevent the penetration of extreme cold on freezing, or great heat when thawing out. The greatest danger to buds from cold, he says, is in the spring after activity has commenced, when a few degrees of frost will often kill the tissue. It is on just such occasions that the frost is likely to be of short duration, and to occur for a few hours only, just before sunrise. It is quite conceivable that the non-conductivity of the scales would be sufficiently great to prevent an extreme fall of temperature within the bud during that short time. Again, if the cold was severe enough actually to freeze

[29] GRÜSS, J., Beiträge zur Biologie der Knospen. Jahrb. Wiss. Bot. **23**: 649. 1892. See also HENSLOW, *l. c.*

the tissues, then when the sunlight fell upon the buds in the morning the scales would prevent injury from too rapid thawing. To support this view he found that shoots of *Picea Engelmanni* deprived of scale-caps perished, while normal ones did not. Shoots of *Betula alba* projecting slightly beyond the sheath were overtaken by a slight frost. The portion beyond the sheath was killed while the protected portion was uninjured. At a temperature of −3° R. the portion of the shoot outside of the sheath in Larix and *Pinus Cembra* was completely killed. A horsechestnut tree at −5° R. had the portion of shoot projecting beyond the scales killed, the rest was uninjured. At −5° R. shoots of this species were killed, while at −4° R. they were all right. Buds of *Acer platanoides* still in the scales were killed at −4° R. Populus cannot stand a cold of −5° R. after breaking out of the buds. Shoots of birch not inclosed were killed at −5° R., but not at −4° R; those still in the bud-scales were uninjured at −5° R.

I am unable to agree with GRÜSS that these cases of protection are due mainly to the modification of the temperature. During the spring of 1902 I removed the scales from a large number of oak buds, also from birch and from maple. This was done about the time that the buds were swelling rapidly; but no frosts occurred afterward until the leaves were quite far developed. Many of the oak buds so treated died, and the rest were retarded or deformed in various degrees. The appearance in all cases was that of drying out—as though the tissue had simply shriveled up from lack of water. The birch and maple showed the same effect though in a less degree. Any one seeing these experiments could scarcely believe otherwise than that the loss of water caused the injury. When the shoot is young the epidermal cells are but slightly cutinized, and are therefore much more pervious to water vapor than after having become more mature. At this period also the cells have probably not yet reached their full osmotic activity, and are still unable to replace rapidly the lost water. It is not surprising, therefore, that shoots should be injured at this period. Even in tropical regions, young tissue is protected against transpiration by being folded within the leaves, or in other ways. GROOM[30] has brought this point out nicely

[30] GROOM, P., Bud protection in dicotyledons. Trans. Linn. Soc. II. **3**:255. 1893.

for temperate regions. He points out that old parts often cover the new. The blade of the young leaf is often covered by stipules. The most critical time is when first exposed, the walls then being thin and feebly cutinized, the chlorophyll dilute and easily decomposed. The blades after coming out are folded and covered with hair in such a way as to diminish transpiration and radiation as well as to reflect light. According to GROOM the function of mucilage and tannin in buds is to help hold the water in the young shoots.

In this light I think we can see the function of the air and wool which gave the negative results in the temperature experiments. Water vapor diffuses through air quite slowly unless the air itself is in motion. If a layer of substance containing air passages such as wool, through which there is almost no circulation, is placed around a damp object, the evaporation from the object is very much retarded because the air in contact with the water is almost saturated under certain conditions.

The horsechestnut wool, therefore, although not functioning in the bud would become a most efficient protection against loss of water from young shoots after leaving the bud-scales. I think this is the purpose of nearly all hairy coverings of young flowers and branches, which view is strengthened by the fact that in most cases the hair disappears before maturity. The putting up of the hair already in the bud insures its presence at the very earliest moment when it shall be required. These and other similar observations have quite firmly convinced me that the growing out of bud-scales and the presence of hair on the young organs is mainly for the purpose of retarding transpiration. In some cases they may be important also for mechanical support.

The observations of GRÜSS in regard to death at temperatures slightly below the freezing point I think can be explained in this way. When tissues freeze the water enters the intercellular spaces and can from there escape more easily to the outer air. If, however, hair or scales were present, not so much water would escape while the tissue was frozen, and a lower temperature might be necessary to cause fatal loss of water. In the case where $-5°$ R. caused death while $-4°$ R. did not, it is possible that freezing just began at that point which is near the over-cooling point of such tissue. It may

be, however, that there are times when the temperature barely falls to the death point, and then only for a very short time just before sunrise, when the bud-scales may save the life of the bud by preventing a temperature fall of from 0.5° to 1° for a very short time, and this little we must admit they are capable of doing. But we must consider that the period when the young shoot is covered by the extending scales, in America at least, lasts only about three or four days at the most, while the probability of a fall just to the critical temperature during this short period is indeed very slight. There may be no frost at all or there may be a very severe one. Only in the case of such a slight frost just reaching the critical temperature could the scales be of benefit, and even this injury would not be fatal to the tree, since another crop of accessory buds can grow out in a short time. The chance to function is therefore very slight, and the effect could not compare in importance to the plant with the benefits obtained according to the above theory, because in the absence of scales nearly all buds of whatever crop would run great danger of being killed by loss of water. It is not reasonable to suppose, therefore, that the benefit derived by modifying temperature is sufficient to have played any great part in causing natural selection to evolve such an elaborate structure for this purpose.

Relation of bud-scales to mechanical injury of the bud.

The idea that the bud-scales serve to protect the delicate young tissues within from mechanical injury is of course not new.[31] In fact, probably everyone feels that this must be, if not the most important function, at least a prominent factor in the work of protection. Nevertheless the subject seems to have received little attention in physiological works.

The young shoots of our definite-growing trees as they exist through the winter are very tender organs, composed mainly of thin-walled parenchyma. In most cases the epidermis is still thin, no fibrous or other supporting tissue has been developed, and the vascular bundles contain only spiral vessels, the result being that they are very brittle and capable of resisting only the slightest concussions. The effect of these abrasions is besides very much inten-

[31] GROOM, P., Bud protection in dicotyledons. Trans. Linn. Soc. II. **3**:255. 1893.

sified by the comparative rigidity of the twig to which the buds are attached. Danger to buds from mechanical causes during the winter may be classified under three heads: danger from birds, from passing objects, and from wind.

Birds.—Young and tender plant tissue is a favorite food for some birds, as for instance the purple finch.[32] It is quite possible that if the bud-scales were absent from our native trees, many species of birds would take advantage of this easy method of obtaining food, at a time when food is scarce. More birds might remain in the North than now, so that altogether it seems probable that trees would suffer severely, if they were not actually killed, by the depredations. A firm hard armor is therefore desirable.

Passing objects.—During my experiments with buds from which the scales had been removed to determine the effect of evaporation, etc., many buds were prepared in a thicket of lilac bushes about six feet high. I found great difficulty in passing through to inspect the buds without breaking off some of them. A moment's absent-mindedness while taking notes would frequently result in the destruction of several buds, a very slight touch only being necessary to dislodge the tender shoot, and the brittleness was of course very much increased when the tissues were frozen. If the above results occurred when care was observed in passing, how much greater would be the damage caused by animals both large and small running thorugh the dense copses in winter. Protective armor seems here again to be a necessity.

Wind.—In our American climate, at least, this is much stronger in winter than in summer—in other words, at exactly the time when buds are frozen and therefore most brittle. The beating together of branches during a heavy wind storm could scarcely fail to do incalculable damage to a tree with unprotected buds. SCHUMANN[33] believed that one of the most, if not the most, important functions of bud-scales is to resist injury from heavy winds. I myself was

[32] FORBUSH, E. H., Birds and woodlands. Mass. State Board of Agric. Rep. **1900**:300. BEAL, F. E. L., How birds affect the orchard. Year Book Dept. of Agric. **1900**:291. ALTUM, Zerstörung von Baum- besonders Fichten- und Kiefernknospen durch Vögel. Zeitschr. Forst. u. Jagdu. **29**:224–230. 1897.

[33] SCHUMANN, C. R. G., Anatomische Studien über die Knospenschuppen von Coniferen und dicotylen Holzgewächsen. Biblioth. Botan. 15:23. Cassel. 1889.

much impressed by the abrasive power of objects beaten about by the wind in winter in the case of firm paper tags attached by strings two inches long to twigs of apricot. During the winter they succeeded not only in marring the bark, but also in completely disintegrating all the buds within reach by simple contact while being blown about. It would seem that the beating together of larger and harder objects like the branches themselves would do even more damage. Therefore, in this case also a firm outer coat is demanded.

I believe that we are justified in concluding from these considerations that mechanical protection is one of the most important functions of the bud-scales—indeed the most important of all.

Some other suggested functions of bud-scales.

GRÜSS[34] included the storing of food material as one of the functions of bud-scales. Bud-scales undoubtedly do store considerable food in some cases, but this is a secondary rather than a primary function. We can scarcely consider this as having been an important factor in the evolution of the scales.

GROOM[35] suggests injury from excess of light as one of the things from which bud-scales protect the delicate young tissue; that when about to unfold the cell walls are thin and the chlorophyll is dilute and easily decomposed. If we conclude that the red color in young plants is for the purpose of modifying the light, then perhaps there is some danger to the young tissues of the bud from too strong light, since these usually become red. No direct evidence however is at hand to warrant this conclusion, and since such protection could be obtained with the expenditure of less energy by the use of hairs or bloom, there seems to be little reason for considering this a determining function of the bud-scales.

One of the most interesting suggestions is that of CADURA[36] to the effect that in addition to protecting the delicate parts from loss of water, great radiation, cold, and too great gas interchange,

[34] GRÜSS, J., Beiträge zur Biologie der Knospen. Jahrb. Wiss. Bot. **23**:648. 1892.

[35] GROOM, P., Bud protection in dicotyledons. Trans. Linn. Soc. II. **3**:255. 1893.

[36] CADURA, R., Physiologische Anatomie der Knospendecken dicotyler Laubbäume. Breslau, pp. 42. 1887.

bud-scales function beneficially by mechanically preventing too early opening of the buds. That buds, as for instance the horse-chestnut and lilac, do open several days earlier when deprived of their scales I have frequently noted in connection with the experiments conducted on desquamated buds in the greenhouse. Still the evidence is not sufficiently strong to warrant the assumption that this is an important and determining function of the scales. Scaleless buds in nature instead of opening very early open quite late, and, as SCHUMANN[37] has insisted, many buds during warm wet autumns open in spite of the scales. My own observations would tend to show that at best they can retard the opening not more than three or four days.

SUMMARY.

Buds containing a considerable number of well differentiated organs are usually protected by scales. Those sunk in the bark usually contain little besides the growing point or rudimentary leaves. Bud-scales are not only the most feasible structures for covering a large bud, but they also allow the bud to swell, and protect the young shoot when unfolding.

The bud fundament in most trees is laid down early in the summer, grows gradually till late autumn, remains dormant until early spring, then passes through a period of swelling preparatory to unfolding.

Dark-colored buds are usually warmer within than light-colored ones; but the question whether there is any relation between dark color and the early opening of the buds was not decided.

Ice may be found in most buds when the temperature has fallen as low as – 18° C. and usually in large quantities. Its absence in other cases is due to small-celled tissues and meager water content.

Frozen twigs are smaller than normal ones. Their contraction occurs mainly in the bark.

Frozen buds do not show this contraction so plainly, probably because of change of form in the bud-scales.

The thawing of buds, if sufficiently slow, is per degree in inverse

[37] SCHUMANN, C. R. G., Anatomische Studien über die Knospenschuppen von Coniferen und dicotylen Holzgewächsen. Biblioth. Botan. 15:27. 1889.

ratio to the freezing, so that at the freezing point of the tissue all the ice will have disappeared.

Regarding the function of bud-scales, there is little evidence that they function by keeping the water out; neither are they important to the plant as modifiers of temperature.

Bud-scales have probably been evolved to prevent excessive transpiration and to protect the delicate tissue from mechanical injury.

When the bud opens the scales often grow out, forming a tube-like structure which protects the young shoot from too great loss of water.

The wool in such buds as horsechestnut is not to modify the temperature, but to protect the young shoot from too great transpiration.

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THE LIFE HISTORY OF POLYSIPHONIA VIOLACEA.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXXIII.

SHIGEO YAMANOUCHI.

THIS preliminary paper will give a brief sketch of my cytological studies on *Polysiphonia violacea* Grev., which were begun last summer at the Marine Biological Laboratory, Woods Hole, Mass., where I occupied a Carnegie Research Table, and were continued at the Hull Botanical Laboratory as a Fellow in the University of Chicago. The problem was suggested by Dr. BRADLEY M. DAVIS, to whom I wish to acknowledge my great indebtedness for his assistance and criticism during the progress of the investigation.

Many points, which for the sake of brevity are omitted in this paper, together with a discussion of literature will be presented in a detailed account with plates to be published later.

METHODS.

The material was killed and fixed in Flemming's fluids, in several modifications which contained the osmic acid in various proportion, Hermann's fluid, 1 per cent. picric acid, and others; among which the weaker formulae of Flemming proved most effective. The best fixation for the study of spermatogenesis and the germination of carpospores and tetraspores was obtained in material killed in weak chrom-acetic acid (Flemming's formula), without any osmic, as follows: 1 per cent. chromic acid, 25^{cc}; 1 per cent. glacial acetic acid, 10^{cc}; sea water, 65^{cc}.

Material was left in the fixing fluid five to forty minutes, and then washed in a gentle stream of sea water. If material remains for a longer time in chrom-acetic acid it becomes very soft and breaks apart. The washed material was passed very gradually through a series of alcohols beginning with 30 per cent., and imbedded in 52° paraffin. The sections were cut 3–5 μ in thickness and stained with safranin-gentian-violet or with iron-alum-haematoxylin, sometimes followed by some plasma stains as orange G, Bordeaux red, Congo

red, or safranin. Preparations were studied with a Zeiss apochromatic immersion 1.5^{mm}, N. A. 1.30, and compensating oculars.

GERMINATION OF THE CARPOSPORE AND TETRASPORE.

It is very easy to obtain the early stages in the germination of carpospores and tetraspores. Fruiting plants, placed in a dish of sea water over night, will discharge great quantities of spores. These fall to the bottom of the dish and germinate at once. The germinating spores may be readily gathered from the bottom at the proper hours to obtain critical stages.

The first division of the carpospores and tetraspores takes place within 10–15 hours after their escape from the parent plants.

The cytoplasm before the first division shows a coarse network or very irregular alveolar structure on the periphery, which becomes much finer around the nucleus. The nucleus has a very delicate membrane, within which lies the linin network, much finer in structure than that of the cytoplasm. The delicate transverse walls of the alveoli of the cytoplasm seem to end on the nuclear membrane where the linin thread starts, which leads the writer to believe that there is some relation between the positions of the walls of the cytoplasmic alveoli and the linin of the nuclear network. The nucleus contains one or two nucleoli homogeneous in structure.

Approaching the prophase of mitosis the linin threads become more and more conspicuous and chromatin granules appear in rows; but without constructing a uniform continuous spirem the threads segment into a number of chromosomes. The nucleus becomes surrounded by dense kinoplasm consisting of very minute closely crowded granules, and the outer margin of this kinoplasmic mass assumes a fibrillar structure which finally ends in the alveoli of the cytoplasm. The distinct concentration of the kinoplasmic masses at the poles to become the centers of the dynamic activities of the mitosis does not occur until the chromosomes are arranged in an equatorial plate. The nuclear membrane persists through the prophase, which makes it evident that the spindle is entirely intranuclear in origin.

The chromosomes at the equatorial plate split longitudinally, and the two groups of daughter chromosomes pass to the opposite poles of the spindle, where they become closely crowded in a mass near the center of the accumulation of kinoplasm.

The nucleoli sometimes fragment into two or three small globules, or decrease in size without fragmentation, finally vanishing during the late prophase. New nucleoli appear after the formation of the daughter nuclei. No such close relation seems to exist between the linin thread and the nucleolus as to warrant a belief that in Polysiphonia the nucleolar substance passes directly into the linin thread to form the chromosomes, as is reported in Nemalion.

No centrosomes could be found during this process of mitosis, although the kinoplasm surrounding the nuclear membrane becomes denser during later prophase and finally accumulates at the poles of the spindle at the time of the metaphase. The masses of kinoplasm present no radiations, yet it seems probable that they function as centers of dynamic activity during mitosis, persisting until the daughter nuclei are organized.

The mitoses within the germinating carpospore and tetraspore correspond in all essentials, except that it became at once apparent in the investigation that the nucleus of the carpospore contained about twice as many chromosomes as that of the tetraspore. Counts of the chromosomes made during the later prophase and metaphase of the mitosis made it clear that the nucleus of the carpospore contains about 40 chromosomes and that of the tetraspore 20.

SPERMATOGENESIS.

The mitoses in the vegetative cell of male or antheridial plants will be described before those of spermatogenesis. During the prophase the chromatin granules increase in size and become grouped as a number of short rod-shaped bodies upon the linin thread, without developing a regular and uniform chromatin spirem. The linin thread then segments into 20 chromosomes.

Polysiphonia, as a rule, is dioecious; however, cystocarpic plants sometimes produce antheridia, a condition which will be described later with other abnormalities. The antheridia develop as lateral branches near the tips of the main filaments. Each branch consists of a central axis from which clusters of sperm mother-cells or spermatocysts arise at the side on short stalk cells.

The cytoplasm of the spermatocyst has a delicate granular structure and contains a large vacuole, generally in the center of the cell. Its nucleus in the resting state is similar to that of the vegetative cell,

and during prophase the linin network becomes more conspicuous and finally segments into 20 chromosomes. The kinoplasm around this nucleus is rather scanty, even after the spindle fibers are formed. The spindles are intranuclear. No centrosome could be found, but there is a concentration of kinoplasm at the two poles.

After anaphase, the nuclear membrane dissolves and the vacuole intrudes into the nuclear cavity between the two sets of daughter chromosomes, one set passing to the apex and the other remaining at the base of the sperm mother-cell. The upper part of the cell, including the vacuole, is then cut off as the sperm from the lower portion by a cleavage furrow, which crosses the cell somewhat obliquely.

The nucleus which remains at the bottom of the sperm mother-cell now repeats this mitosis, forming a second sperm, and perhaps two or three more are developed before the antheridium ends its fertility.

OOGENESIS AND FERTILIZATION.

The mitoses in the vegetative cells of the female or cystocarpic plant are similar to those of the male. The number of chromosomes is invariably 20.

The female organ or procarp develops from a central axial cell, next to the apical cell of a short lateral branch. The central axial cell gives rise to a pericentral cell from which by successive mitoses a four-celled carpogonial branch is formed. The terminal cell of this series becomes the carpogonium, situated as a rule above the pericentral cell, owing to the curved growth of the carpogonial branch.

The carpogonium is at first round and the nucleus lies in its center. While this nucleus undergoes a typical mitosis to form two nuclei, the carpogonium puts forth the process which is to become the trichogyne. After mitosis one of the nuclei proceeds into the developing trichogyne to become a trichogyne nucleus, and the other remains below in the carpogonium as the gamete nucleus.

Coincident with the development of the trichogyne, the pericentral cell gives rise to the two sets of auxiliary cells, one of which is a series of three or four, formed above, so that they lie just beneath the carpogonium, and the other series consists of two cells formed below.

When the sperm comes in contact with the trichogyne, the walls

between dissolve, the contents of the sperm cell enter the cytoplasm of the trichogyne, and the sperm nucleus passes down into the carpogonium where the fusion of the male and female nuclei takes place.

The trichogyne nucleus may be found even after the sperm nucleus has passed into the carpogonium. But later, when the trichogyne becomes separated from the carpogonium, its nucleus can scarcely be distinguished. The trichogyne then shrivels and dies.

The carpogonium after fertilization unites with one of the auxiliary branch cells which lies beneath, and the latter also fuses with the pericentral cell, thus providing a passage into the pericentral cell for the fusion nucleus of the fertilized carpogonium. Then the remainder of the auxiliary branch cells fuse with one another and with the pericentral cell, which results in a large fusion cell, the central cell, that naturally contains a number of nuclei.

The nuclei in the central cell are of two sorts with respect to origin: first, there is the fusion nucleus from the carpogonium (sporophytic); and second, there are a number of nuclei derived from the auxiliary cells, which are of course gametophytic. The fusion nucleus gives rise to a series of nuclei by typical mitoses which present 40 chromosomes as a sporophyte number. The central cell now develops several lobes into which these sporophytic nuclei pass. Further mitoses increase this number, and each lobe then cuts off a carpospore terminally, which is attached to the central cell by a short stalk.

After the carpospores are formed, the central cell increases in size greatly, absorbing the stalk cells, and even the central axial cell also becomes involved in this general cell fusion. These very extensive cell unions are probably concerned with the nourishment of the carpospores.

Some of the gametophytic nuclei derived from the auxiliary cells remain in the central cell, increasing in size and finally breaking down after a number of peculiar changes. Others of the gametophytic nuclei divide amitotically to form the paranematal filaments which lie under the wall of the cystocarp.

TETRASPORE FORMATION.

The tetrasporic plant normally never produces antheridia or procarps, and the cytological studies on the vegetative cells give proof that it differs in an important respect from the sexual plants. The

mitoses in growing regions of the tetrasporic plant show that the nuclei have 40 chromosomes (the sporophyte number), while it will be remembered that the nuclei of the sexual plants have 20.

I shall not enter at this time into a detailed description of the events which take place during the formation of the tetraspore mother-cell; the only thing to be remembered is that the number of the chromosomes appearing during this mitosis is 40, so that it follows that the nucleus of the tetraspore mother-cell contains 40 chromosomes.

The nucleus of the tetraspore mother-cell increases somewhat in size, accompanied by the growth of the cell itself; yet the latter is relatively slow until just before the first mitosis of the nucleus, but very rapid after that.

The resting nucleus of the tetraspore mother-cell contains a fine network of linin in which the chromatin is distributed irregularly in larger and smaller granules. The nucleolus has no visible connection with the linin thread. With the further growth of the nucleus the linin thread increases in thickness; in such an irregular way, however, that in some parts the threads are uniform in thickness and in the others they appear to have knots. The chromatin thread now forms a fairly well-developed spirem.

This condition presently passes into the so-called stage of synapsis, when the spirem consists visibly of two parallel threads close together, while in the other parts the two are in contact side by side or fused into a single thread. The two threads may represent, according to recent interpretations of synapsis, chromatin of maternal and paternal origin.

After synapsis, the tangled thread becomes distributed throughout the cavity of the nucleus. The spirem now shows the longitudinal fission which precedes the separation of chromatin granules into two sets, and then the spirem segments into 20 chromosomes, each showing clearly its bivalent nature.

While this process of chromosome formation is going on in the interior of the nucleus, the kinoplasmic material surrounding the nucleus becomes concentrated at two poles of a spindle, and when the chromosomes are arranged in the equatorial plate a minute body occupies the center of each pole. The body might be called a centrosome, but it has not been possible to recognize its presence during prophase or to follow it after anaphase.

It is interesting to note that the two poles are not in a straight line perpendicular to the center of the equatorial plate, but are asymmetrical and less than 180° apart. Each of the 40 sporophytic chromosomes composing the 20 pairs (bivalent chromosomes) arranged in the equatorial plate now splits longitudinally, so that a large number of univalent chromosomes results, probably 80 in all, although I was unable to count the exact number.

The group of 80 grand-daughter chromosomes separates into two sets, but resting neuclei are not formed, and their further distribution by the second mitosis begins at once. The axes of the two spindles of the second mitosis lie perpendicular to each other, and their complex relation to that of the first will be described in the final paper. Kinoplasmic masses occupy the poles of the spindles in the second mitosis, and each contains a centrosome-like granule.

Now, each group of 40 chromosomes, following this first mitosis, separates into two sets of grand-daughter chromosomes, 20 in each set, which are attracted toward the respective poles of the two spindles, where the kinoplasmic material becomes more pronounced than before. These chromosomes, after reaching the four poles, become massed together, lose their individual outlines, and larger and smaller granules appear shortly after on linin threads which later become contained in the four daughter nulcei.

It is a remarkable fact that the membrane of the original nucleus in the tetraspore mother-cell persists through the two mitoses which have just been described. The area included by this membrane increases in size with the growth of the cell. The cytoplasm around it shows larger alveoli, which become smaller in the vicinity of the original nuclear membrane, and at last passes into the kinoplasmic fibrils immediately surrounding it.

At this time constrictions appear simultaneously in the area marked by the original nuclear membrane. The kinoplasm intrudes into this area in a very interesting manner, which cannot easily be described without figures, but results in the organization of the four daughter nuclei that are to be contained in the tetraspores.

The division of the tetraspore mother-cell does not take place simultaneously with the events described above. Cleavage furrows start along four lines on the periphery of the cell, a little before the end of the nuclear division, and proceed more rapidly after its com-

pletion. During the entire process of the tetraspore formation, the mother-cell is connected by a strand of protoplasm with the stalk-cell, and probably gets much nourishment through this strand, for the tetraspore mother-cell increases greatly in size. Finally, the cleavage furrows meet in the center between the four daughter nuclei, thus dividing the protoplasm into four tetraspores.

ABNORMALITIES.

Normally, the male and female organs and the tetraspore are never developed in the same plant, but it often happens that the male and female organs are produced on the same individual, and occasionally antheridia are formed on the same branch with procarps and cystocarps. The sexual cells in these cases are developed normally, the number of the chromosomes being always 20. Cystocarpic plants have also been found producing cells whose lineage is identical with that of the tetraspore mother-cell. However, I have never seen evidence of nuclear division in such a cell; cleavage furrows appear and cut deeply into the protoplasm, which nevertheless remains undivided, so that tetraspores are not formed. Whether this cell may escape and germinate as a monospore has not yet been determined.

CONCLUSION.

The nuclear conditions in the life history of Polysiphonia may be summarised as follows:

1. The germinating carpospore contains 40 chromosomes, and the tetrasporic plant the same number; so it may be inferred that the tetrasporic plants come from carpospores.

2. The germinating tetraspore contains 20 chromosomes, and the sexual plants (gametophytes) the same number; so it may be inferred that the sexual plants come from tetraspores.

3. The nuclei of the gametes (sperm and carpogonium) contain each 20 chromosomes. The fusion nucleus (sporophytic) in the fertilized carpogonium presents 40 chromosomes, and gives rise to a series of nuclei. Some of these enter the carpospores, which are consequently a part of the sporophytic phase to be continued in the tetrasporic plant. The gametophytic nuclei in the central cell of the cystocarp (with 20 chromosomes) either break down or form the paranematal filaments.

4. Tetraspore formation terminates the sporophytic phase with typical reduction phenomena, so that the tetraspores are prepared to develop the gametophytic generation.

5. There is thus an alternation of sexual plants (gametophytes) with tetrasporic plants (sporophytes) in the life history of Polysiphonia, and the cystocarp forms a part of the sporophytic phase.

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THE STRUCTURE AND DEVELOPMENT OF THE BARK IN THE SASSAFRAS.

HOWARD FREDERICK WEISS.

(WITH NINE FIGURES)

THE common sassafras occupies a somewhat isolated position among northern trees. It is not only the single living representative of the genus Sassafras, but it belongs to the Lauraceae, a family with many arboreal genera in tropical and subtropical regions, but with very few in the cooler parts of the earth. The tree is further remarkable because its young branches remain green for a considerable period, differing in this respect from the majority of the trees among which it grows. For these various reasons it was hoped that a study of the bark might reveal features of interest.

MÖLLER has already studied the bark in several genera of the Lauraceae and has included in his published account a short description of what he found in the sassafras.[1] According to his researches the family as a whole is characterized by the following peculiarities in the bark: a late appearance of cork; an epidermal origin of the phellogen; a slight development of collenchyma in the outer cortex, most of the cells remaining thin-walled and parenchymatous; the occurrence of stone-cells in the medullary rays between the strands of primary sclerenchyma; the presence of ethereal oil and slime in some of the parenchyma cells; the scattered bast fibers in the inner or secondary bark. With regard to the sassafras in particular he notes that the cork is homogeneous and composed of thin-walled cells and that the inner bark is destitute of stone cells. It should be remarked that most of MÖLLER'S material in this family consisted of dried bark, much of which was fragmentary and in poor condition.

In his more general account of the Lauraceae SOLEREDER accepts the majority of MÖLLER'S statements with regard to the bark.[2] Quoting from J. E. WEISS, however, he notes the fact that the phellogen is not invariably epidermal in origin, but that it is sometimes derived

[1] Anat. der Baumrinden 103–110. 1882.

[2] Syst. Anat. der Dicot. 795. 1899.

from the layer of parenchyma just within the epidermis. He also remarks that the secondary bast fibers, although usually scattered, form distinct strands in certain genera, and that the individual fibers are normally four-sided in cross section with narrow lumina.

The present investigation is based on material collected near New Haven, Connecticut, and is confined to the stem and its branches, no reference being made to the bark of the root. The tissues described may be classified as follows:

PRIMARY TISSUES	SECONDARY TISSUES
Epidermis	Tissues derived from the cambium ring
Outer cortex	The phellogen and its derivatives
Primary medullary rays	
Primary bast	

PRIMARY TISSUES.

Epidermis.

The epidermal cells are characterized by a strongly thickened cuticle. Close to the growing point they are isodiametric and thin-walled, but the cuticle begins to make its appearance very early and practically completes its development during the first year's growth. At the close of this period it occupies about half the thickness of the epidermis (*fig.* 1). During the elongation of the stem the epidermal cells retain the power of growth and division. Since their growth is largely in a longitudinal direction, the cell-division is mainly brought about by transverse walls, division by longitudinal walls being much

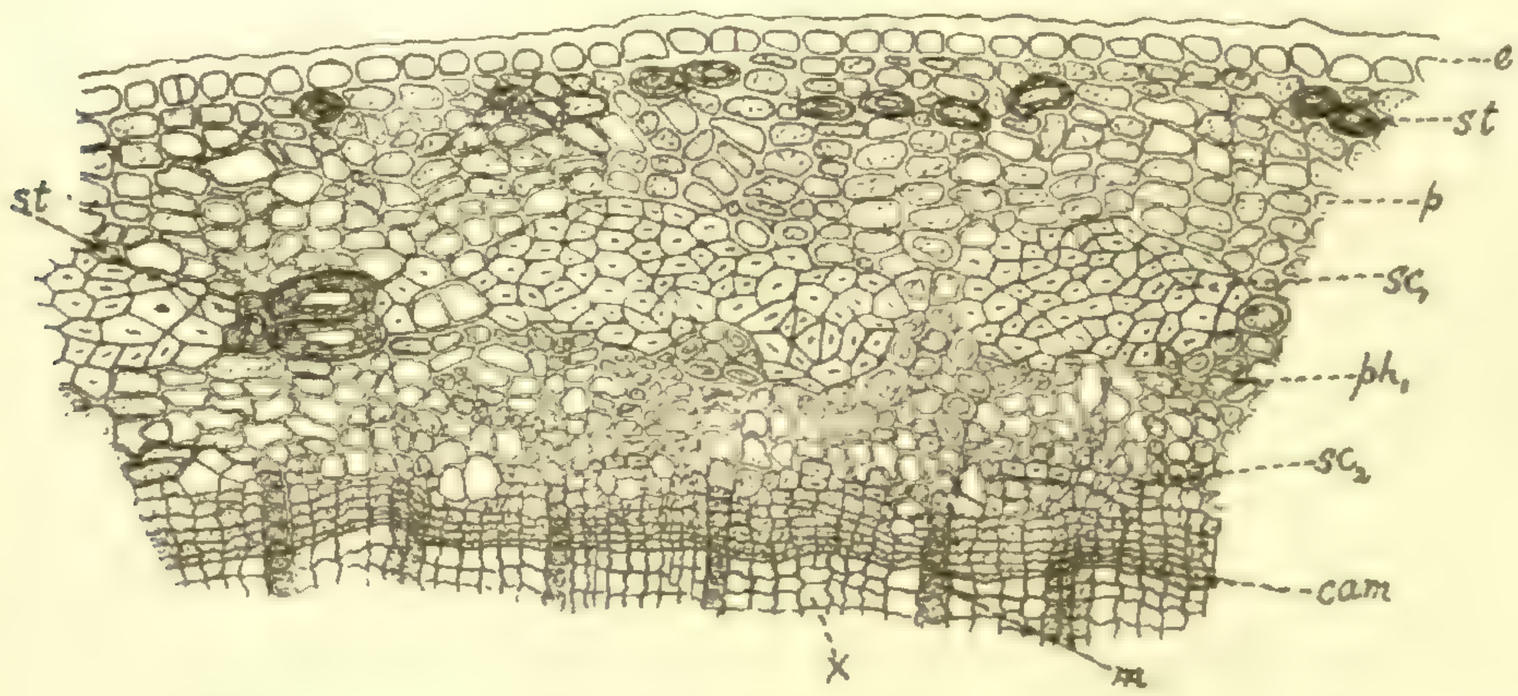


FIG. 1.—Cross-section through bark one year old. ×70. *cam*, cambium ring; *e*, epidermis; *m*, medullary ray; *p*, parenchyma; ph_1, primary phloem; sc_1, primary sclerenchyma; sc_2, secondary sclerenchyma; *st*, stone cells; *x*, xylem.

more infrequent. In an epidermis a year old, seen from the surface, the boundaries of the original epidermal cells can usually be distinguished. They are somewhat thicker than the secondary transverse walls, which in turn are thicker than the secondary longitudinal walls (*fig. 2*). With the formation of cork the epidermis is of course split longitudinally and soon begins to undergo disorganization. No trace of it is left in a tree 8^{cm} in diameter.

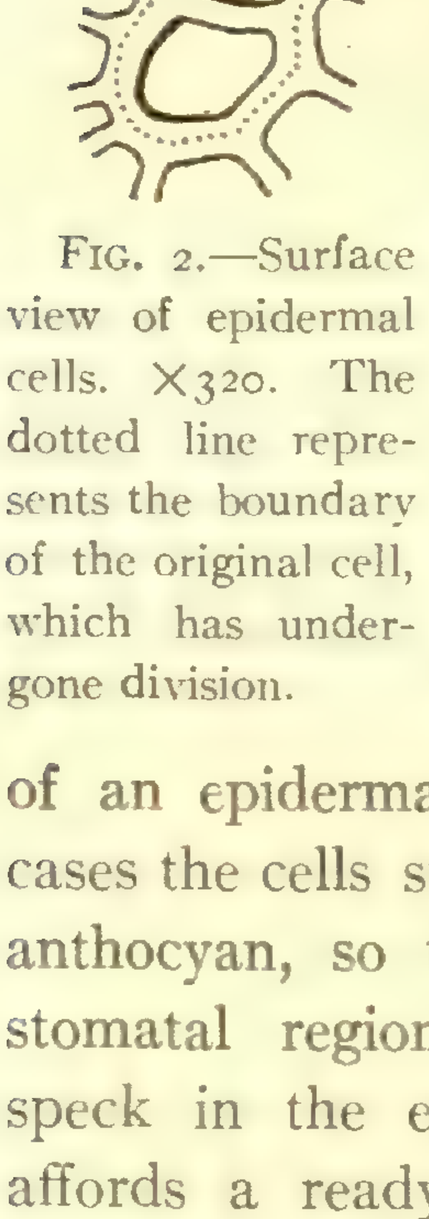


FIG. 2.—Surface view of epidermal cells. ×320. The dotted line represents the boundary of the original cell, which has undergone division.

The number of stomata produced varies greatly, but seems to be largely dependent upon external conditions. A rapidly growing tree, for example, in a moist locality has many stomata, while a slow-growing tree in dry soil develops very few. The stomata are depressed and the epidermal cells bounding the guard cells are somewhat modified, being longer and narrower than their neighbors (*fig. 3*). Most of the stomata are transverse to the axis upon which they are borne, a few are oblique, but apparently none of them occupy a longitudinal position. This is doubtless to be explained by the fact that the stomata are formed late in the development of the epidermis, the wall separating the guard cells representing one of the secondary transverse divisions of an epidermal cell. In the majority of cases the cells surrounding a stoma contain anthocyan, so that to the naked eye the stomatal region looks like a minute red speck in the epidermis. This peculiarity affords a ready means for detecting the stomata.

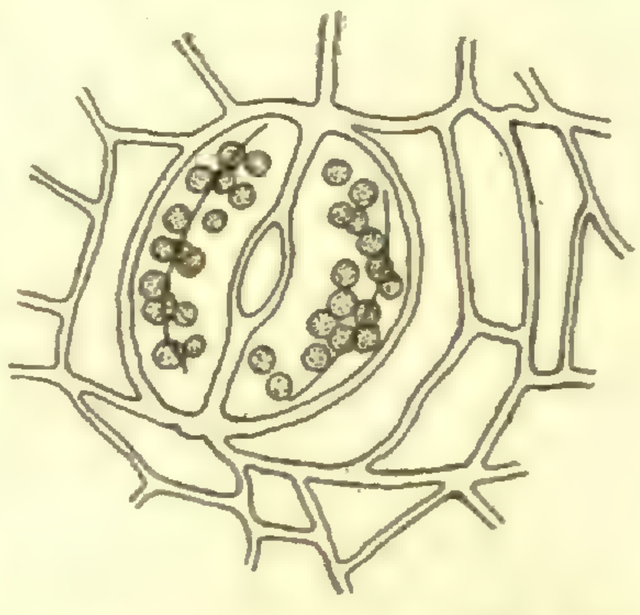


FIG. 3.—Stoma with surrounding cells, surface view. ×320.

Epidermal hairs are developed on very young twigs before the primary tissues are fully differentiated. They are simple and unicellular, with thickened walls, and scarcely extend below the cuticle (*fig. 4*). These hairs never persist through the first vegetative period, but dry up and fall away as soon as the cuticle begins to thicken. Their former position is often marked by

small concave depressions in the cuticle. The number of the hairs varies, and in a general way is inversely proportional to the number of stomata. Thus, in a moist locality few hairs are formed, while in a dry region they are very abundant. In a mesophytic area some trees bear few hairs, while others under the same conditions bear very many. It would appear from this that the production of hairs was primarily due to individual peculiarities of the tree in question and secondarily to the external conditions under which the tree developed.

FIG. 4.—Epidermal hairs on a twig one month old. ×50.

Outer cortex.

The outer cortex comprises everything external to the primary sclerenchyma except the epidermis. It is composed of a ground mass of parenchyma with scattered stone-cells. No crystal cells occur. With the formation of cork the outer cortex gradually becomes disorganized and eventually disappears.

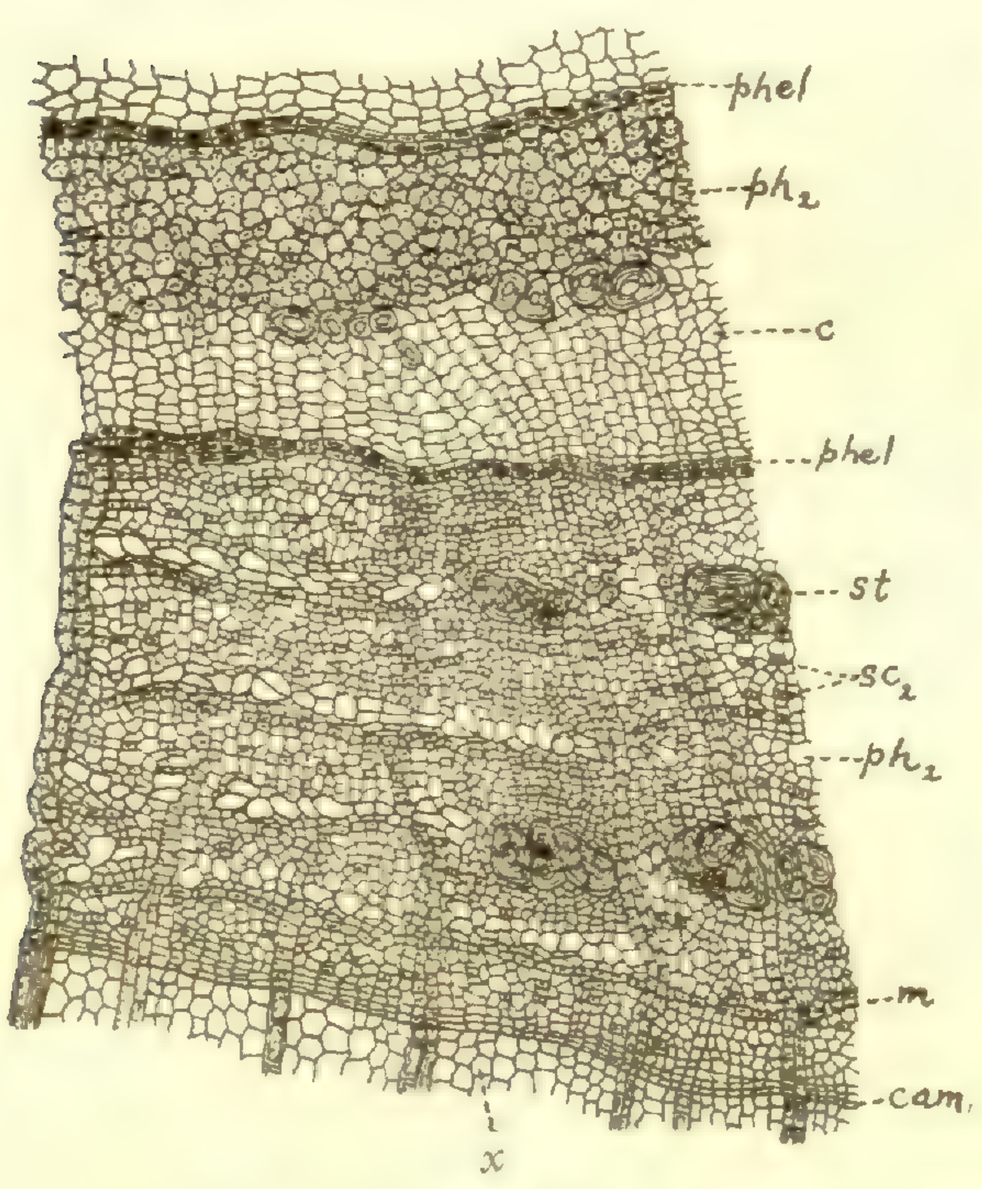
In cross section the parenchyma cells vary from elliptical to rectangular in outline, the long diameter running in a tangential direction (*fig.* 1, *p*). They vary considerably in size and some of the larger cells have their walls slightly lignified. Most of the cells, however, have thin walls, which may or may not be provided with simple pits. Many of the smaller cells contain starch and this is especially likely to be true of those which border the strands of sclerenchyma. The presence of ethereal oil in the parenchyma can be demonstrated by appropriate tests, but it does not seem to be localized in special cells. In all probability the oil represents an excretory product of the protoplasm of the parenchyma cells, and this fact would account for its general distribution.

The stone cells form a continuous or interrupted layer extending entirely around the stem (*fig.* 1, *st*). They sometimes lie next to the epidermis and are sometimes separated from it by one or two layers of parenchyma cells. The stone cells are at first circular in cross section but afterwards become flattened and assume an elliptical outline. In radial section they appear rectangular, being about three times as long as broad. Their walls are strongly thickened by deposits of

ligno-cellulose in distinct layers, and these are pierced by numerous simple and branched pits.

Primary medullary rays.

The primary medullary rays extend from the cambium to the outer cortex, the ray cells merging into the cortical cells without a distinct line of demarcation. The outer portion of the ray is of course directly differentiated from the meristem at the growing point, while the inner portions owe their existence to the activity of the cambium. Some of the cells in the outer portion retain their power of growth and division for several years, the majority of the dividing



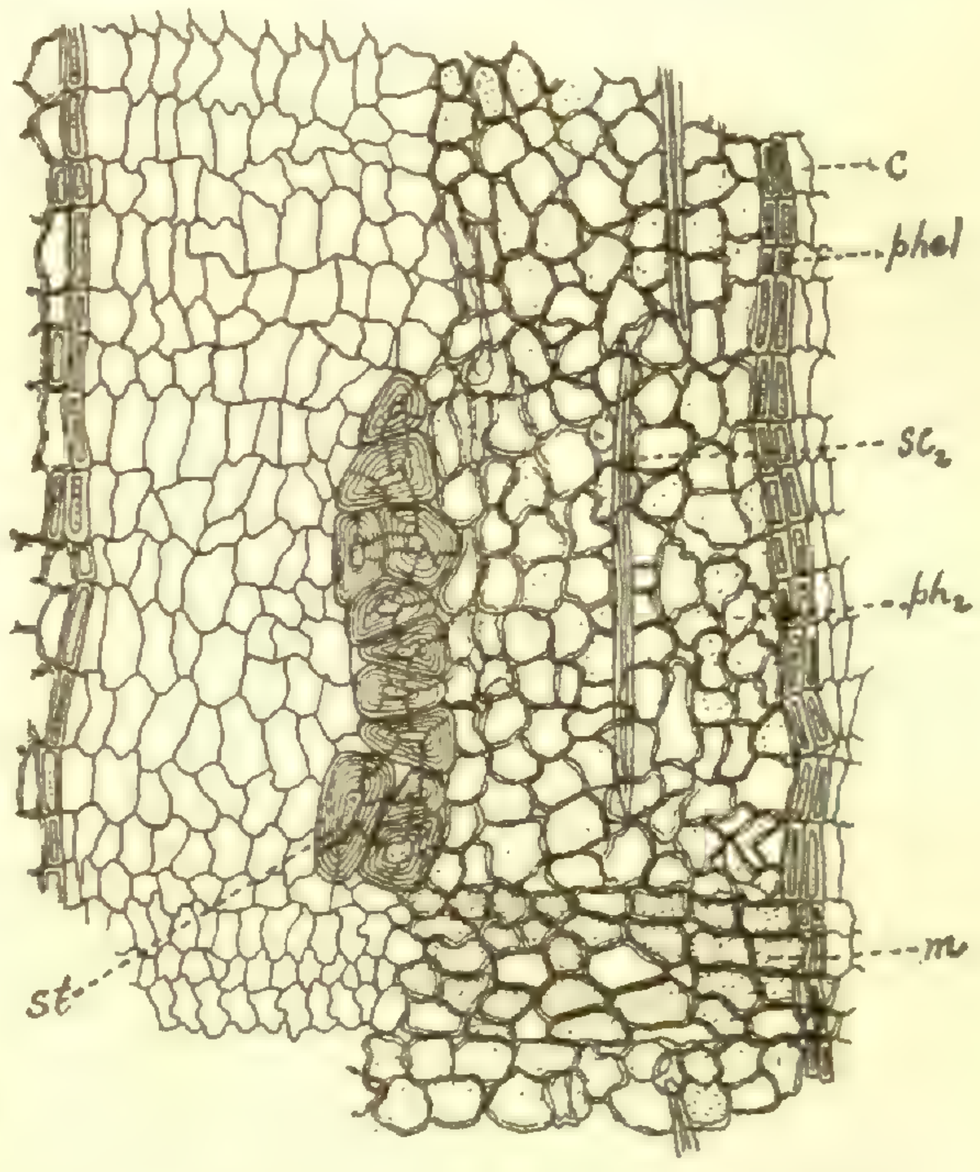


FIG. 5.—Cross section through old bast. ×25. FIG. 6.—Radial section through old bast. ×75. *c*, cork; *cam*, cambium ring; *e*, epidermis; *m*, medullary ray, *p*, parenchyma; ph_2, secondary phloem; *phel*, phelloderm; sc_2, secondary sclerenchyma; *st*, stone cells; *x*, xyiem.

walls being radial. Thus, in a stem one year old, the strands of primary sclerenchyma are separated by from two to five layers of cells, in a stem two years old by as many as fifteen layers, while in a stem four years old the number may be increased to thirty or more. Since the portions of the rays derived from the cambium do not undergo further divisions, they remain permanently from one to three cells in width. In consequence of these facts the rays gradually assume a T-shape

in cross section. This form is retained until the outer cortex has become disorganized, after which they appear like narrow bands (*fig. 5, m*). In radial section the rays are from four to fourteen cells across (*fig. 6, m*).

In most of the ray cells the walls are slightly thickened and provided with numerous simple pits. They usually contain starch and sometimes ethereal oil as well. When the cells are cut off by cork the starch disappears, showing that it is completely utilized; the oil, on the other hand, persists. Some of the ray cells between the strands of primary sclerenchyma become strongly sclerotic, and in some cases cells of this character completely bridge the space from one strand to another (*fig. 1*). They can be easily distinguished from the sclerenchyma cells, even in cross section, by their larger size and distinct lamination. In longitudinal section they appear short and resemble the stone cells of the outer cortex.

Primary bast.

The primary sclerenchyma occurs in well-defined bundles, averaging about fifty fibers apiece (*figs. 1, 8, sc_1*). Most of these bundles, in a radial direction, measure from three to eight cells across. In most of the fibers the wall is so strongly thickened that the cavity is reduced to a mere slit; in some cases, however, the thickening is less and this is especially likely to be true in the middle of a bundle. Apparently the deposition of ligno-cellulose upon the cell walls is not completed until the second vegetative period.

The primary phloem lies just within the primary sclerenchyma, between the latter and the secondary sclerenchyma, and forms a band from three to five cells across in a radial direction (*fig. 1, ph_1*). The sieve tubes are more or less completely separated from the sclerenchyma by a layer of phloem parenchyma. The cells of this layer tend to be rectangular in cross section, and their slightly thickened walls have numerous simple pits. The sieve plates separating the segments of the sieve tubes are nearly always somewhat oblique; they are supplemented by numerous lateral sieve plates, especially in the radial walls of the tubes. All of the sieve plates in the primary phloem soon become covered by deposits of callus. The companion cells conform to the usual type.

SECONDARY TISSUES.

Tissues derived from the cambium ring.

The tissues of the bark, regularly derived from the cambium ring, include the secondary sclerenchyma and the secondary phloem. In addition to these, scattered groups of stone cells, which should probably be considered a part of the phloem, also make their appearance. Of course the cambium also adds new elements to the primary medullary rays and brings about the development of the secondary rays (*figs. 1, 5*). The development of these various secondary tissues begins during the first vegetative period.

The fibers of the secondary bast do not form bundles. Some of them form interrupted layers arranged concentrically in the stem, others are scattered through the secondary phloem. The layers are usually but a single cell across and are separated from one another by several layers of phloem. The individual fibers are rectangular in cross section and about thirty times as long as broad; their walls are very strongly thickened (*figs. 1, 5, 6, sc_2*). When the bast fibers are cut off by cork all regularity in their arrangement disappears.

The sieve tubes of the secondary phloem, except those earliest formed, are arranged in interrupted, concentric layers, one or two cells across (*fig. 5, ph_2*). Many of the sieve tubes are in direct contact with the medullary rays, but very few of them adjoin the sclerenchyma fibers. The tubes exhibit essentially the same structure as those in the primary phloem. On account of their delicate walls they become practically indistinguishable when cut off by cork.

The bulk of the secondary phloem is composed of parenchyma. When first differentiated from the cambium the cells of this tissue are closely packed together, rectangular in outline, and destitute of intercellular spaces. As they become pushed outward, their outlines become more rounded and minute intercellular spaces appear. Their walls are fairly thin but are provided with simple pits. Until they are cut off by cork the parenchyma cells are arranged in layers, which lie among the layers of sclerenchyma and sieve tubes.

The groups of stone cells are irregularly scattered in the secondary bast but always abut against a medullary ray (*fig. 5, st*). Such a group in cross section is often larger than a bundle of primary sclerenchyma and is composed of larger elements. The stone cells are the

most conspicuous structures found in the inner bark, and are even more striking in appearance than those found in the outer cortex. In longitudinal section (*fig. 6, st*) they show the same outlines as in cross section (*fig. 7*) and are therefore isodiametric. Their strongly thickened walls show a very distinct lamination and their contracted cavities are connected by numerous simple and branched pits. Probably on account of poor material, these stone cells were not seen by MÖLLER.

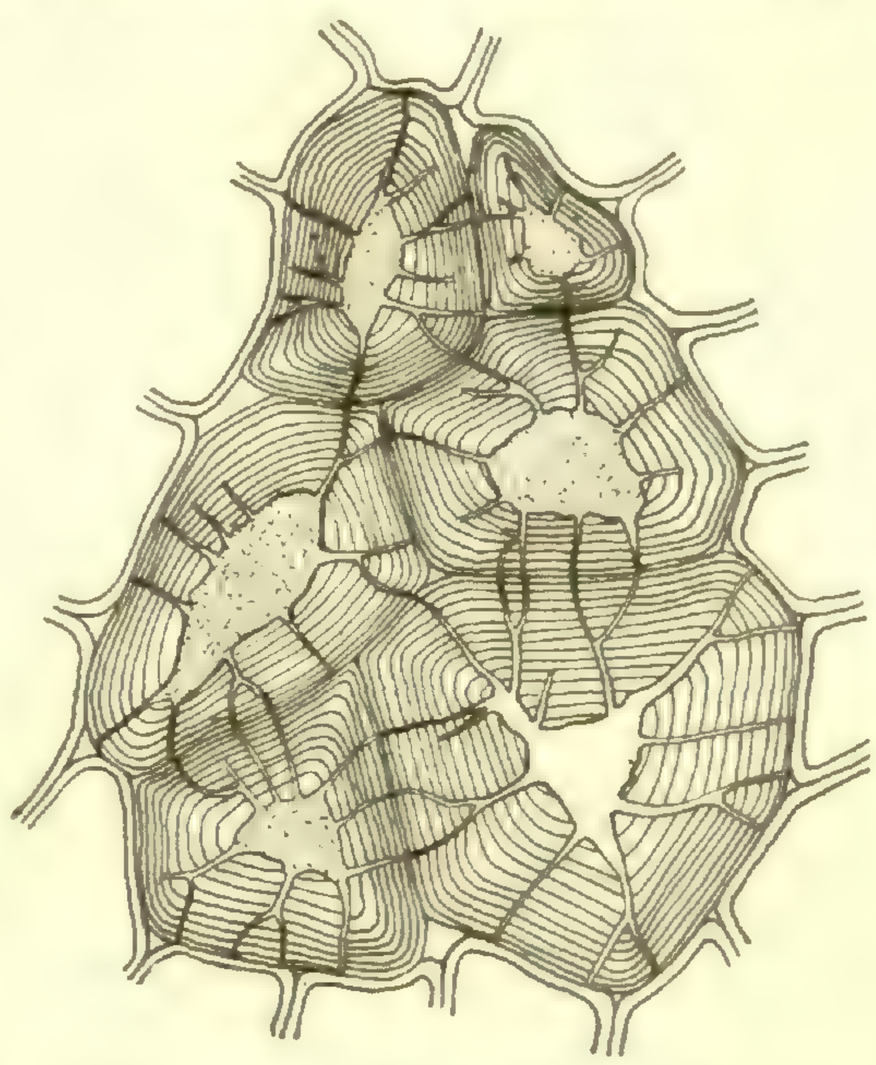


FIG. 7.—Stone cells from inner bark, cross section. ×450.

The phellogen and its derivatives.

The derivatives of the phellogen are the lenticels, the cork, and the phelloderm. The lenticel phellogen is the first to make its appearance; the primary cork phellogen is, at least in part, a direct extension of the lenticel phellogen; and the succeeding phellogens arise more or less independently from the deeper layers of the bark. The primary cork phellogen first appears on the south side of an erect stem and normally on the upper surface of a horizontal branch. From these regions it gradually extends laterally and usually forms a complete layer in the course of three or four years. The development, however, follows no definite rule. For example, in one eight-year old stem there was no cork on the north side except in the immediate vicinity of the lenticels, while in another stem of the same age there were five layers of cork on the south side and three on the north. These observations show that a phellogen layer may be active in one part although it has ceased to be functional in another. They also show that there is no definite relationship between the age of the stem or branch and the number of layers of phellogen. The early appearance of cork in the regions exposed to the sun is probably due to the fact that the sassafras is an intolerant species and that the cork protects the deeper tissues from sun scalding.

The primary lenticels are always formed directly beneath the stomata, following in this respect the general rule first enunciated by TRECUL.[3] Some of the lenticels never break through the epidermis but remain in an undeveloped condition. The lenticel phellogen arises from the layer of cells just within the epidermis. The thin-walled complementary cells are at first closely packed together. After about twelve layers of these cells are formed the epidermis is ruptured, and the complementary cells as they become exposed separate from each other and present very irregular and distorted outlines. The mature lenticel agrees with the second of the types described by DEVAUX[4] and shows no distinct layers of cork among the complementary cells (*fig. 8*). In some cases, however, a lenticel contains a few scattered stone cells (*fig.* 8, *st*). Secondary lenticels are developed from secondary phellogens and make their appearance in the splits of the bark. These lenticels break through the layers of cork and parenchyma cells which enclose them and eventually exhibit the same structure as the primary lenticels (*fig. 9*).

FIG. 8.

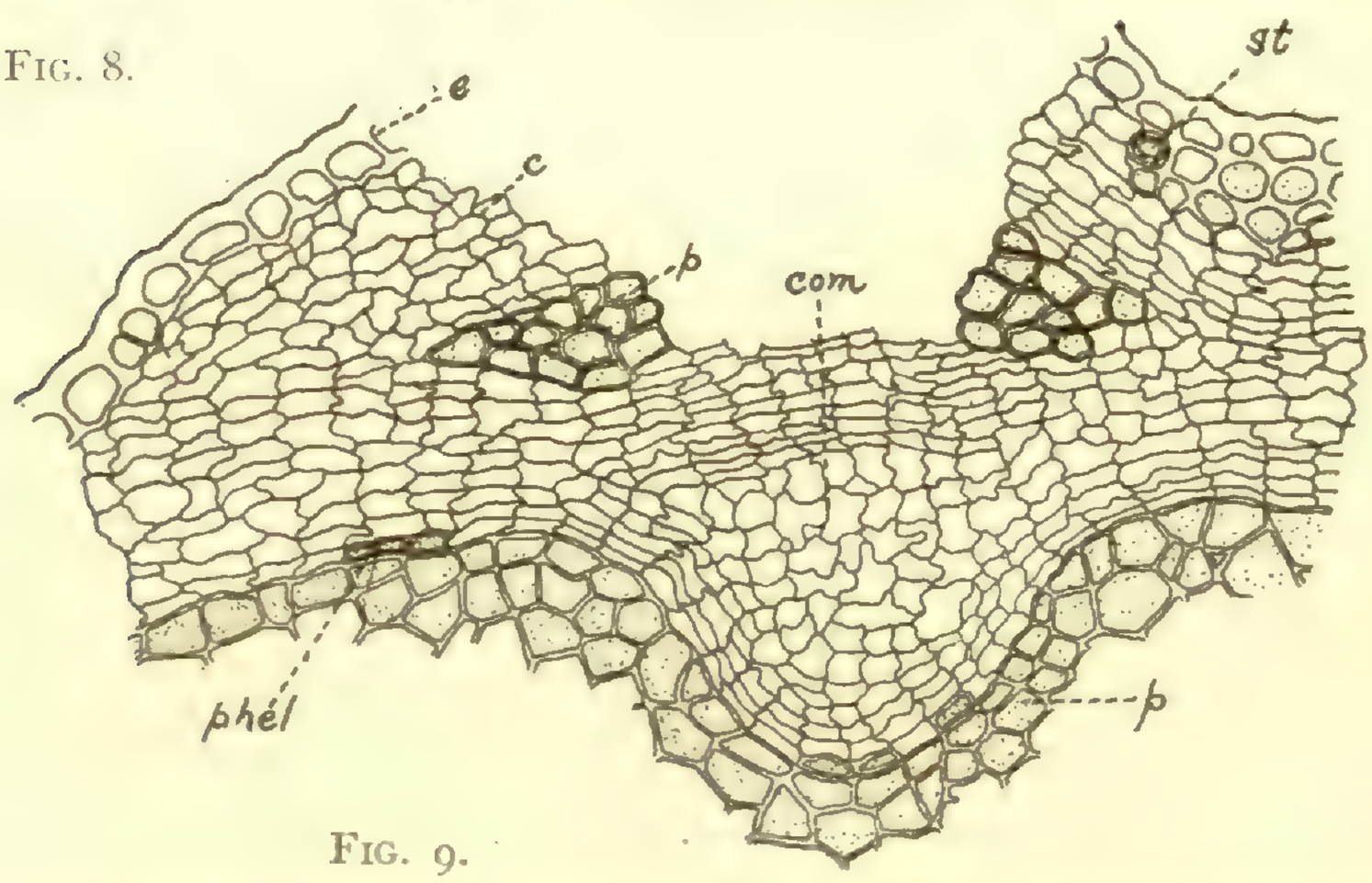


FIG. 9.

FIG. 8.—Section through a primary lenticel. ×55. FIG. 9.—Section through a secondary lenticel. ×60. *c*, cork; *com*, complementary cells; *e*, epidermis; *p*, parenchyma; *phel*, phelloderm; sc_1, primary sclerenchyma; *st*, stone cells.

Since the primary cork phellogen is a direct extension of the lenticel phellogen, it is never epidermal in origin but is always derived

[3] Compt. Rend. **73**:15. 1871. [4] Ann. Sci. Nat. Bot. VIII. **12**:61. 1900.

from the subepidermal parenchyma. In the majority of cases it arises from the layer of cells just inside the epidermis. Sometimes, however, it is derived from the second, third, or fourth layer, and this is always the case when stone cells are present next the epidermis. It thus frequently happens that the different parts of the phellogen do not all arise from the same layer of cells. The secondary layers of phellogen are largely derived from the parenchyma cells in the secondary phloem. When stone cells are present in the parenchyma the phellogen often bounds them on the inside. The phellogen forms concentric layers in the stem, but these layers are not altogether independent. In certain regions two layers will coalesce, in other regions they will be separated from each other by several layers of cells. Even the outermost of the secondary phellogens is more or less united with the primary phellogen.

The cork, as already noted by MÖLLER, is of the ordinary type. It consists of empty cells arranged in radial rows, and the walls are thin and suberised (*figs. 5, 6, 9, c*). In most cases from ten to twelve layers are formed by each phellogen. The structure of the cork is not uniform throughout the Lauraceae; in certain genera it consists of two kinds of cells arranged in more or less definite layers; namely, thin-walled cells and cells in which the inner tangential walls are thickened.[5]

The phelloderm in the sassafras forms a most characteristic feature of the bark. When derived from secondary phellogens it consists almost entirely of strongly flattened cells with thick lignified walls, provided with simple and branched pits. The flattening is in a radial direction, and the cells show the same rectangular outlines in both radial and transverse sections (*figs. 5, 6, phel*). The phelloderm is arranged in layers from one to three cells thick. The layer derived from the primary phellogen differs from the others in being composed of both thin-walled and thick-walled cells. In the case of lenticels the thick-walled phelloderm cells are few and scattered and are sometimes absent altogether. Lignified phelloderm does not seem to be of very frequent occurrence. According to J. E. WEISS[6] it is to be found in species of Cytisus and Philadelphus; KUHLA[7]

[5] See MÖLLER, Anat. der Baumrinden 103. 1882.

[6] Beiträge zur Kenntniss der Korkbildung. Denkschr. Königl. Bayer. Bot.Gesells. **6**:61. 1890.

[7] Bot. Centralbl. **71**:196. 1897.

describes it for *Ptelea trifoliata*, and SOLEREDER[8] notes its appearance in several genera of the Saxifragaceae other than Philadelphus. It therefore occurs in widely scattered families and probably has but little taxonomic significance.

SUMMARY.

Among the more interesting points brought out by this study are the following: the early thickening of the cuticle; the variation in the number of epidermal hairs and stomata; the early formation of cork in regions exposed to the sun; the stone cells in the outer bark, between the strands of primary sclerenchyma, and in the inner bark; the regular layers of thick-walled phelloderm derived from the secondary phellogens.

The writer is indebted to Professor ALEXANDER W. EVANS for criticism and advice.

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[8] Syst. Anat. der Dicot. 360. 1899.

BRIEFER ARTICLES.

THE DISTRIBUTION AND HABITS OF SOME COMMON OAKS.

When doing some work in Wisconsin last year for the Arnold Arboretum, I found that *Quercus ellipsoidalis* E. J. Hill was well represented in the woods of the southeastern part of that state. It was originally described from trees growing in the vicinity of Chicago. It had been identified by those studying the flora near Milwaukee, and is quite abundant on the hills of the Kettle Range. It had also been recognized as distinct by those unfamiliar with botanical works, as disclosed by the common name "pin oaks." I had not before heard this name applied to any except *Q. palustris* Moench. The original description mentioned the usual but not universal drooping of the lower branches, as is quite common in the pin oaks. When finding it in some new locality I have sometimes been at a loss to decide which of the two species it was till the acorns were in hand. As the branches often come low down, they are apt to die as the trees grow older, and, breaking off a short distance above their base, leave stubs along the trunk, so characteristic of the pin oaks. This was freely the case in most of the trees seen in Wisconsin, and doubtless explains the local name.

Quercus palustris was not seen in any of the localities visited, nor did I learn of its presence from those familiar with the flora. In 1846 Dr. Lapham mentions its occurrence at Milwaukee in a book containing "A list of plants which have not before been noticed as indigenous to Wisconsin."[1] It was mentioned again by him in a paper on the "Plants of Wisconsin."[2] Though no locality is specified, it is understood from a prefatory statement to have been "within thirty miles of Milwaukee." If rightly identified (and Dr. Lapham was a careful and competent observer), it would seem to have disappeared. Yet there is the possibility that the tree with drooping lower branches with stubs along the trunk, and finely divided leaves, going by the common name of pin oak, was the one he alluded to, since the common name is added to the botanical in both of the above citations.

That botanists have been bothered by some form ascribed to *Q. palustris* or *Q. coccinea* is apparent from a statement of Dr. George Vasey in an

[1] Wisconsin: its geography and topography, history, geology, mineralogy, etc. Milwaukee, 1846, p. 73.

[2] Proceedings of American Association for the Advancement of Science 1849:19.

article on "Our native oaks." "It (*Q. palustris*) is found in low and swampy ground, and in general appearance much resembles the scarlet oak (*Q. coccinea*), and perhaps may yet have to be considered a variety of that polymorphous species."[3] Dr. VASEY resided for several years in northern Illinois, and could hardly have failed to see such forms of *Q. ellipsoidalis* as have led to its being confounded, by the common people at least, with the pin oak. But a typical scarlet oak is a tree of quite a different aspect from *Q. palustris*, and from its habitat would be more easily confounded with *Q. ellipsoidalis*. It is true that *Q. palustris* is commonly confined to low ground, though not always swampy, as along the margins of streams which have cut their beds deep down into the drift, leaving a high bank. Here the pin oak holds its place on ground that trends away from the stream and is comparatively dry. I have seen it along the Kankakee River move out of a swampy area to a bordering locality where the limestone was but a few inches below the surface. And although *Q. ellipsoidalis* commonly grows on dry or upland ground, it also occurs in lower, even wettish, localities, as by the borders of ponds and sloughs in low woods, becoming a near neighbor of the swamp white oak (*Q. platinoides*). Those seen in Wisconsin were on hills of till, or by the borders of lakes in the Kettle Range, or in soil of glacial drift. The least frequent of the biennial-fruited oaks associated with it seemed to be *Q. velutina*. *Q. coccinea* was quite common; *Q. rubra* the most abundant of all. In Illinois I have most frequently met with it in woods adjacent to streams not subject to overflow, the morainal hills being taken, when wooded, more by *Q. coccinea*, *Q. rubra*, *Q. velutina*, and *Q. imbricaria*, in prevalence somewhat in the order given.

It is therefore a matter of some doubt whether *Q. palustris* now occurs in Wisconsin. In Minnesota it is mentioned in UPHAM'S "Catalogue of the plants of Minnesota" on the authority of Dr. LAPHAM, the locality not being given; and on the authority of another collector as found in the region of the Upper Mississippi. I have not been able to get these statements verified. The pin oak of Minnesota may also be *Q. ellipsoidalis*. Professor SARGENT identifies this in specimens collected at the Falls of Minnehaha in 1878, and states that he himself first saw the species in 1882 at Brainard on the Red River of the North and at St. Paul.[4] In his report on the forest trees of North America, tenth census, volume 9, *Q. palustris* is given for Wisconsin; but in his account of the tree in the eighth volume of the *Silva*, this state is omitted from its range, as well as in his more recently published *Manual of the trees of North America*. Both Wisconsin

[3] The American Entomologist and Botanist **2**: 376. 1870.

[4] Silva of North America **14**:50. 1902.

and Minnesota are rather far north for its range. The farthest north I have found it in Illinois is in the town of Niles, just north of Chicago. Nor can I find any record of its occurrence in the more northerly counties of the state, where, if occurring at all, it is evidently very scarce. Dr. FRIEDRICH BRENDEL of Peoria, in an article on "The trees and shrubs of Illinois," says "The pin oak (*Q. palustris* Du Roi) I have never seen around Peoria, nor did, as I learn by letter, Mr. HALL in Menard County; it occurs in St. Clair and Marion Counties; in Wisconsin and Cook County (*fide* Mr. JACKSON)."[5] The credit to Wisconsin is doubtless due to Dr. LAPHAM, already cited. South of Chicago this oak appears in the southern part of Cook County in the town of Thornton, extending sparingly up Thorn Creek for a short distance, where it grows in company with *Q. ellipsoidalis*. It is most abundant east of the village of Thornton, making a good part of a wood growing in a soil of sandy peat, patches of sphagnum being common under the trees. Eastward it is found in occasional spots and in similar soils, and in the clayey soils of swamps in Lake and Porter Counties, Indiana. It comes into the dune region of Lake Michigan north of the village of Porter, in a sandy humus soil similar to that near Thornton.

Southward from here in Indiana it increases in frequency and abundance. In eastern Illinois it reappears south of the Thorn Creek localities after one crosses the range of hills here forming the water-shed of Lake Michigan basin (the Valparasio moraine), and is frequent along the Kankakee River at Momence.

Whether *Q. ellipsoidalis* occurs south of the most northern counties of Indiana there is no evidence at hand to show. Some time spent in examining the flora in the vicinity of North Judson and English Lake in Stark County did not reveal its presence, though the pin or Spanish oak was common along the Kankakee River there. Specimens of oaks sent from Bluffton in the eastern part of the state, a short distance south of Fort Wayne, lacked this species, but contained *Q. palustris* and *Q. texana* Buckley.

It is evident from this survey that *Q. ellipsoidalis* replaces to a large extent in the north of the Middle West the more southerly *Q. palustris*. But it is usually with a different and drier habitat, and an adaptability to a wider range of conditions. The boundaries of the two overlap in southern Michigan, northern Indiana, northern Illinois, eastern-central Iowa, possibly in southern Wisconsin. It may also be of interest to add that the northern bounds of another biennial fruited oak, the shingle oak (*Q. imbricaria*) correspond quite generally with those of *Q. palustris*.—

E. J. HILL, *Chicago*.

[5] Illinois Agricultural Report 1859:596.

CURRENT LITERATURE.

BOOK REVIEWS.

Botanical dictionary.

In 1900 Jackson published the first edition of his *Glossary of botanic terms*, and last fall the second edition appeared.[1] We welcomed the first edition[2] as being a marked improvement upon any existing dictionary, and criticized but lightly the most obvious shortcomings. The compiler, most competent in many respects, had certain limitations by reason of his unfamiliarity with the content and consequently the terminology of morphology and physiology, and our general criticisms lay along these lines.

In judging the second edition one looks to see whether this weakness of the first has been removed, either by the author's own efforts, or by his associating with himself those who could supply the lacking knowledge. We find that the "revised and enlarged" of the title page means only that typographical and minor errors have been corrected in the plates of the first edition, and that a supplement of 68 pages has replaced the former "Additions during printing." One can overlook much in a first edition that cannot be forgiven in a second. Perhaps there will be a third with a resetting that will allow the necessary improvement. In that hope we may point out certain objectionable features that should receive attention.

In the first place it would be desirable to relegate to a separate list the many terms which have become obsolete, most of which are adopted from Lindley's *Glossary* and were antiquated in his day. Technical language changes rapidly, and such terms should be put into a museum and labeled as exhibits, if shown at all. We should then escape reading (except we were on antiquarian research bent) that an *ovule-tube* is "a thread-like extension of the amnios, rising beyond the foramen;" and, when we turn in wonder to see what the *amnios* in plants could have been, learning that it is "a viscous fluid which surrounds certain ovules at an early stage." We do not need often to know that *prosphyses* were "abortive pistillidia of the muscal alliance," and the youngster who has occasion to look for the word should learn that both it and its definition are mere survivals from a past century.

Second, space could be gained by omitting to define common words which have no technical meaning, such as congeries, enlargement, entangled, evapora-

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[1] Jackson, Benjamin Daydon, A glossary of botanic terms, with their derivation and accent. Second edition, revised and enlarged. 12mo. pp. 371. London: Duckwith & Co. (Philadelphia: J. B. Lippincott Co.) 1905.

[2] Bot. Gazette **31**: 68. 1901.

tion, minute, parallel, sex, tall, wound. All of these and many others are now included.

Third, more care should be taken to make definitions sufficiently general to include the various uses of the word, rather than so special as to refer only to particular uses. Thus, *conjugate* appears as an adjective, but not as a verb; *conjugating tubes* are defined in a special and unusual sense for the Rhodophyceae and not at all for the Conjugatae; for *pistil* is given (after a wholly erroneous definition in reference to spermatophytes) an obsolete sense which is restricted to the genus Andreaea, when in the same sense it was formerly applied to the archegonia of all mosses; *retardation* is not mentioned as other than the "influence of light on growth in certain structures;" and a *fat enzyme* is defined merely as an enzyme "converting olein into oleic acid and glycerin."

Fourth, greater accuracy is sadly needed. A few examples will illustrate this: *Galvanotropic*, "curvature, etc.;" *geotropism*, "the force of gravity as shown by curvature;" *geotaxis*, "movement in plants caused by gravity;" *stamen*, "a male sporophyll;" *pistil*, "the female organ of the flower;" *staminate*, "applied to flowers which are wholly male;" *oogenesis*, "the formation of the oosphere, the early stage of the ovule" (but oosphere is correctly defined later in the same paragraph!); *sap-pressure*, "the force exerted on passing upwards through the tissues;" *spermatogenesis*, "the development of the male elements, antherozoids, pollen-grains, and analogous bodies;" and so on.

Fifth (a matter for the publisher), the use of a more flexible paper and looser binding would contribute much to the handiness of the volume.—C. R. B.

MINOR NOTICES.

The dynamics of living matter.[3] —In the spring of 1902 Professor JACQUES LOEB was invited to deliver a series of lectures at Columbia University. In these lectures, eight in number, he presented the gist of his researches upon the dynamics of living matter. This book, forming the eighth volume of the Columbia University Biological Series, is a somewhat more complete survey of the field of experimental biology, says the author, than was possible in the lectures. In ten "lectures" he discusses the general chemistry and physical constitution of living matter, certain physical manifestations of life, the rôle of electrolytes, effects of radiant energy, heliotropism and other tropisms, fertilization, heredity, and regeneration.

Through the publication of his collected papers in English in the Decennial Publications of the University of Chicago[4] Professor LOEB's point of view and the general results of his experimentation have become even more generally

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[3] LOEB, J., The dynamics of living matter. Columbia University Biological Series VIII. 8vo. pp. xii+233. *figs. 64*. New York: The Columbia University Press. 1906. $3.

[4] LOEB, J., Studies in general physiology, 1905.

known than from the originals. The topics named above are naturally those with which the author has chiefly concerned himself, and it cannot be said that the present volume contributes to general physiological literature anything new. The book is rather a new setting of the brilliant work and suggestive ideas of the author, that have previously enriched physiology, and with them is related the results of others in such wise as to round out the presentation. The lectures are readable and instructive, and they are especially commended to the attention of plant physiologists, who are too apt to pass over literature not strictly pertaining to plants.—C. R. B.

The problems of life.—The third part of this book[5] was issued last winter, and extends the author's fundamental hypothesis to the phemonena of fertilization and heredity. To him, if one admits the premises, the difficulties of these phenomena fade away like morning mists. The work does not cite definite observations, nor show, except in the most general way, how the known facts can be correlated by this theory; but it presents a clearly reasoned, logical series of deductions, which impresses the reader at once as too simple to be true. Moreover, one is naturally shy of a theory, which, beginning with an assumption regarding the molecular structure of protoplasm and the nature of assimilation, makes reproduction a necessary and inevitable consequence of these assumptions, while heredity likewise follows as a matter of course from the phenomena of fecundation. We were inclined to welcome the molecular conceptions of the first part,[6] as possibly embodying a fruitful theory, but we cannot follow the author as he widens and heightens his construction upon the acute fundamental assumption. Such inverted pyramids of logic can have no stability.—C. R. B.

Pfeffer's Physiology.—The third and last volume of this work was published about the middle of March.[7] It treats at length of the movements of plants, including the mechanical responses to various stimuli; and briefly of the production of heat, light, and electric tensions, and of the sources and transformations of energy. The translation, or rather the interpretation of the original, is of the same satisfactory character as in earlier volumes. As before, the editor has introduced supplementary and critical matter in footnotes; and in an appendix of eight pages he has supplied some important facts not mentioned in the first two volumes, and a summary of the more recent literature, especially that connected with the present volume. Throughout, his critical

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[5] GIGLIO-TOS, ERMANNO, Les problèmes de la vie. IIIe partie: La fécondation et l'hérédité. 8vo. pp. viii + 189. Cagliari: The author, at the University. 1905. *fr.* 8.

[6] Cf. BOT. GAZETTE 31:275. 1901.

[7] PFEFFER, W., The physiology of plants, a treatise upon the metabolism and and sources of energy in plants. Second fully revised edition; translated and edited by ALFRED J. EWART. Volume III. Imp. 8vo. pp. viii + 451. *figs.* 70. Oxford: The Clarendon Press. 1906. 21*s.*

care and acumen have enriched the already valuable work of the author, so that English readers are indebted to him for far more than a translation of peculiarly difficult German. To recommend the English form to all libraries and laboratories as a standard work of reference is, at this date, really quite superfluous.—C. R. B.

British flowering plants.—Under this title Lord AVEBURY,[8] better known as Sir JOHN LUBBOCK, has brought together a mass of desultory notes on various things connected with a great many plants. The author says that this work is "to describe points of interest in the life-history of our British plants; to explain, as far as possible, the reasons for the structure, form, and color; and to suggest some of the innumerable problems which still remain for solution." A glossary and an introductory chapter indicate that the book may be used by those with no botanical training; and perhaps it will be chiefly so used. Each species is taken as the occasion for the presentation of all sorts of facts and fancies and questions in reference to it, as though the author had emptied his note book under that head. There is no distinct organization and no adequate index; so that the botanist will simply have to "run on" to things.—J. M. C.

Spring flora of Ohio.—Under the title "Spring Flora," the botanical staff of Ohio State University has issued a manual for beginners and amateurs.[9] It is a revised edition of KELLERMAN'S "Spring Flora of Ohio," and its range has been extended so as to include Ohio and Indiana and the adjacent states. The time range extends from the opening of the season into the first part of June; and such difficult groups as grasses and sedges are not included. There is also a key to the trees and shrubs based on leaf and twig characters.—J. M. C.

Flora of Norway.—AXEL BLYTT'S completed *Handbook of the Norwegian Flora*, including the vascular plants, has been issued under the editorship of OVE DAHL.[10] In reality it has been in preparation since 1861, having been begun by the father, continued by the son, whose name is on the title page, and now finally edited by a third botanist. It is a model of compact and clear printing, excellent arrangement, and good text figures. The sequence is that of Engler and Prantl.—J. M. C.

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[8] AVEBURY, THE RIGHT HON. LORD, Notes on the life history of British flowering plants. 8vo. pp. xxiii+450. *figs.* 352. New York: The Macmillan Company. 1905. $5.00.

[9] KELLERMAN, W. A., GLEASON, H. A., and SCHAFFNER, J. H., Spring flora for beginners and amateurs. pp. xiii+188. Columbus, Ohio: Geo. W. Tooill. 1906. 75 cents.

[10] BLYTT, AXEL, Haandbog i Norges Flora. Efter forfatterens dod afsluttet og udgivet ved OVE DAHL. pp. xi+780. *figs.* 661. Kristiania: Alb. Cammermeyers Forlag. 1906.

Portraits of botanists.—In 1903 WITTROCK published a set of photographs of botanists selected from the collection at the botanical garden at Stockholm. A second series has now been issued,[11] containing full-page portraits of 100 botanists arranged chronologically from Aristotle to Goebel; and 51 additional plates, each containing 6 portraits. The biographic notes contain a large amount of information which must have been brought together with great labor.—J. M. C.

British Desmidiaceae.—In 1904 the first volume of this work was issued as a publication of the Ray Society. The second volume has now appeared,[12] containing the genera Euastrum (46), Micrasterias (18), and Cosmarium (50). —J. M. C.

NOTES FOR STUDENTS.

Regeneration.—The number of recent papers dealing with regeneration indicates a marked activity in this field of investigation. The work of IRMISCH and others has made us familiar with the fact that the hypocotyls of a number of plants can produce adventitious buds. In some cases these occur normally, but in others only in the presence of more unusual conditions of growth. BURNS and HEDDEN[13] have investigated these conditions, using seedlings of *Linaria bipartita splendida*, *Antirrhinum majus*, and *Linum usitatissimum*. They confirm KÜSTER'S results that when the cotyledon or the main vegetative tip is cut away the tendency toward the development of adventitious buds is greatly increased. On uninjured seedlings of Antirrhinum which do not stand erect but are horizontal, buds arise only on the upper side, and when these plants are fastened so that they must remain erect they produce no buds. The effect of a moist atmosphere is to increase the number of buds and the rapidity of their development. The same is true of higher temperature. The older parts of the hypocotyl have a much greater capacity to produce buds than the younger parts, and there is no tendency at all to bud production on the part of the hypocotyl still elongating. Gravity seems to have no influence. Light, on the other hand, is a necessary condition, for in one-sided illumination buds appear only on the illuminated side, on a klinostat in the light on all sides equally, and in the dark not at all. Experiments are mentioned which indicate that wounding is not a cause of the regeneration here. The explanation of the phenomena mentioned as given by the authors is that "when the cotyledons are removed

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[11] WITTROCK, VEIT BRECHER, Catalogus illustratus iconothecae botanicae horti Bergiani Stockholmiensis; notulis biographicis adjectis. Acta Hort. Berg. 3:No.3. pp. xciii+245. *pls. 151.* 1905.

[12] WEST, W., and G. S., A monograph of the British Desmidiaceae. Vol. II. pp. x+206. *pls. 32.* London: Ray Society. 1905.

[13] BURNS, GEORGE P., and HEDDEN, MARY E., Conditions influencing regeneration of the hypocotyl. Beih. Bot. Centralb. 19:383–397. 1906.

or cease to function, their work is taken up by the epidermis. The cells of this develop a vast amount of chlorophyll and all movement is to and from them.'' "Only those cells exposed to light function as cotyledons, and hence all flow of material is to and from the lightest side. Light is then an indirect cause of the location of the buds, while the principal factor is determining the location in relation to movements of food materials in plants." This would make it entirely a question of nutrition, a rather unusual condition, for in most cases of regeneration in plants, and in animals too, regeneration will occur while the parts concerned are but poorly nourished.

FIGDOR[14] cut off the apices of young fern fronds prependicular to the median axis and very close to the tip, removing only a fraction of a millimeter of the tip. Replacement occurs slowly, but the new tips become forked, two apical cells forming, one on each side of the midrib. The two sides extend outwards, leaving the midrib sunken in the center. By cutting very young fronds with a median longitudinal cut about 5^{mm} deep, regeneration of each half occurs, and a subsequent branching of the frond is obtained. The fern used was *Scolopendrium Scolopendrium*, a variety of which (*daedalea*) occasionally occurs in nature with forked fronds, and FIGDOR considers this probably due to wounding of the tips by insects and subsequent regeneration.

HILDEBRAND[15] has continued his studies on regeneration in Cyclamen, and presents further interesting observations. Two forms are mentioned, *Cyclamen Miliarakisii* and *C. creticum*. On the former, when the leaf blade of the cotyledon is removed, leaving the petiole, there arise a little below the place of removal, from a point on one side of the petiole, four small leaves, each having the form of the cotyledon, and the four together aggregating the size of the blade removed. Each is borne on a distinct petiole of sufficient length to bring the blades out far enough to prevent shading each other. In this the author sees an exceptional example of the principle of utility in the development of plant structures. In the other species, *C. creticum*, HILDEBRAND observed a plant having no cotyledons, but upon which, arising from the center of the tuber, were three leaves with long petioles. Each blade was almost one-third the size of the round cotyledon-blade, and in form intermediate between the cotyledon and the foliage leaves. Investigation showed that the cotyledon had been destroyed to the base, and these three leaves arose together from the axis of the plant just below the point of attachment of the cotyledon. They originated as entirely new structures, replacing cotyledons, and were intermediate in form between these and the later leaves.

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[14] FIGDOR, W., Ueber Regeneration der Blattspreite bei *Scolopendrium Scolopendrium*. Ber. Deutsch. Bot. Gesell. **24**: 13–16. 1906.

[15] HILDEBRAND, FRIEDRICH, Ueber eine eigentümliche Ersatzbildung an einem Keimling von *Cyclamen Miliarakisii* und einem anderen von *Cyclamen creticum*. Ber. Deutsch. Bot. Gesell. **24**: 39–43. 1906.

SETCHELL[16] gives an account of regeneration among kelps. He distinguishes between physiological and restorative regeneration, applying the terms in the same sense as used by MORGAN. In physiological regeneration he notes two kinds, continuous and periodic. In the former the continuous growth of the meristematic tissue at the base of the blade keeps pace with the constant breaking off at the tip due to wave action, and so the blade retains a constant length. In other species this growth is periodic, occurring in the spring and in the autumn. The growth of a new blade lifts the old one from the top of the stipe and it is rapidly eroded, the new one thus taking its place. Restorative regeneration involves the development of new branches and occurs as a result of wounding. If the stipe is broken off a new blade is formed at its apex. Wounds along the surface of the stipe result in new blades arising at the points. A vertical wound at the tip results in a splitting of the blade and the appearance of forking. The observations are followed by a discussion in which the author contends that the phenomena of regeneration are to be explained best by the assumption of a flow of materials toward the parts concerned. He does not consider it necessary to assume a special organ-forming material, the important thing being the control of the flow of already organized food materials. This control of the food substances is due to certain cells being able to exert a stronger "pull" upon them than others.

As this idea is so commonly used in explanation of regeneration, the reviewer cannot forbear remarking that it removes one difficulty only to incur a greater one. Soluble food materials, in common with all other diffusible solutions in plants, move toward the region of least concentration, and if there is a more rapid flow of substances toward any region, it indicates that these are being taken out of solution there, either by being used or otherwise transformed. The more active the use, the lower will be the concentration, and the more active the flow will tend to be toward that point. The increased activity of the cells, either in using up by growth or otherwise transforming the food substances, must precede any special flow (that is, apart from a general diffusion in all directions) of these substances into any particular region. The movement, or, if preferred, the "flow" of soluble substances (other than a general diffusion) toward special cells is necessarily a result and not the cause of their activity.

MIEHE[17] has used an interesting method of studying the behavior of isolated cells, especially in their relation to polarity. When a tissue is plasmolyzed, the continuity of the protoplasm is broken and the protoplasts become separated from one another. In this way a plant may be divided into its individual cells, and the behavior of these, each acting independently, can be studied. MIEHE used this method on a marine Cladophora. The plants were plasmolyzed in

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[16] SETCHELL, WILLIAM ALBERT, Regeneration among kelps. Univ. Calif. Publ. Bot. **2**:139–168. *pls. 15–17.* 1905.

[17] MIEHE, HUGO, Wachstum, Regeneration und Polarität isolierter Zellen. Ber. Deutsch. Bot. Gesell. **23**:257–264. *pl. 4.* 1905.

a strong salt solution (16.2%), and then transferred gradually to normal sea water. In nearly all cases the protoplasts regained almost entirely their original size, a few remaining in the plasmolyzed form. An active growth promptly set in, by which the form of the alga was entirely changed. First the protoplasm of the last-mentioned cells, by means of rounded or tube-like outgrowth, finally filled up the original space within the cell walls. Then all the cells grew in this way: the basal end of the cell pushed into the cell below in the form of tubes, often growing between the protoplasm and the cell wall; or occasionally the whole cell bulged into its neighbor. When one cell is dead, the next above grows in and fills it completely. Often from the lower angles of the cells tubes grow downward into the cells below. Many of these tubes assume the character of rhizoids. All of these outgrowths occurred at the basal end of the cell, not a single one from the apical end. Later, unless the upper cells begin to produce branches, they do so entirely from the apical end. A very striking polarity of the cell is thus seen.

Some interesting results on polarity and organ-formation on *Caulerpa prolifera* have been contributed by JANSE.[18] This plant he shows to possess a well-marked polarity in the formation of "leaves" and rhizoids, and also in the streaming of the protoplasm, which is always from the apex toward the base. Following a wound there appears to be a division in the protoplasm, the chlorophyll-bearing portion separating from a colorless turbid portion. It is the latter, according to JANSE, that occasions the formation of new organs. The polar phenomena he considers dependent upon a flow of energy in which the force acts always in the direction of the base. This stream of energy he calls the "basipetal impulse." The opposite, an acropetal impulse, was not to be detected, and JANSE concludes that "the lack of an 'acropetal impulse, implies the lack of a second pole at the organic tip." Thus we have a polarity with only one pole. The author applies this conception to polarity as seen in the higher plants. The point of view is more interesting than convincing.

TOBLER[19] uses some observations on Polysiphonia and Ceramium as the basis of a lengthy and rather elusive discussion on regeneration and polarity. He sees a difference in the lower and higher plants in respect to polarity, which he considers rests on the differentiation of tissues, and accompanies the division of labor in the plant.—W. B. MACCALLUM.

Roots of Monocotyledons.—LINDLINGER[20] has reopened the question of the place of origin of the secondary growth shown by the roots of some mono-

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[18] JANSE, M. J., Polarität und Organbildung bei *Caulerpa prolifera*. Jahrb. Wiss. Bot. **42**:394–460. *pls. 9–11*. 1906.

[19] TOBLER, FR., Ueber Regeneration und Polarität sowie verwandte Wachstumsvorgänge bei Polysiphonia und andern Algen. Jahrb. Wiss. Bot. **42**:461–502. *pls. 12–14*. 1906.

[20] LINDLINGER, L., Zur Anatomie und Biologie der Monocotylenwurzeln. Beih. Bot. Cent. **19**:321–358. 1905.

cotyledons, such as Dracaena. Contrary to the usually accepted view, the author finds that cambial activity is present not in the pericycle but in the inner layers of the cortex. Cell divisions in the pericycle are confined to the points where lateral roots make their appearance, and this growth may have been confused by other investigators with the true secondary growth in the cortex. The second part of the paper is devoted to an account of the so-called "Aussenscheide," a zone of more or less thick-walled cells found in the inner cortex of many monocotyledonous roots. This zone is not a real secondary tissue, *i. e.*, produced by the division of meristematic cells, though it may assume the power of secondary growth. This zone is considered to be equivalent to the secondary tissues discussed in the first part of the paper. Several suggestions are offered as to the function of the "Aussenscheide," varying with the habits of the plant in which it occurs. Naturally the mechanical function seems to be the most common one.—M. A. CHRYSLER.

Morphology of Cucumis sativus.—TILLMAN[21] has investigated sporogenesis and embryogeny in the cucumber. The most interesting items reported are as follows: the presence of two integuments that elongate greatly and invest the remarkable beak-like prolongation of the nucellus; the somewhat irregular development of the embryo; and an haustorial enlargement of the pollen tube on its passage through the long nucellar beak. The fusion of the unequal polar nuclei was seen, but no case of double fertilization was observed.—J. M. C.

Enzymes of Polyporus.—BULLER[22] finds in the juice of *P. squamosus* the following: laccase, tyrosinase, amylase, emulsin, a protease, lipase, rennetase, and coagulase; but negative results were obtained by tests for pectase, maltase, invertase, trehalase, and cytase. Yet the fact that it destroys the wood of *Acer pseudoplatanus* indicates the presence of cytase and possibly hadromalase.—C. R. B.

Limiting factors.—An illuminating paper on *Optima and limiting factors* has been published by Dr. F. F. BLACKMAN,[23] which it behooves every physiologist to read. The argument shows, and it is sustained by the results of research, that much physiological experimentation has been falsely interpreted.—C. R. B.

Photosynthesis extra vitam.—MACCHIATI replies[24] to BERNARD,[25] criticizing his methods, maintaining that photosynthesis does occur *in vitro*, and stating certain modifications of the process. He makes a weak case.—C. R. B.

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[21] TILLMAN, OPAL I., The embryo sac and embryo of *Cucumis sativus*. Ohio Nat. **6**:423–430. *pls. 29–30*. 1906.

[22] BULLER, A. H. R., The enzymes of *Polyporus squamosus* Huds. Annals of Bot. **20**:50–59. 1906.

[23] Annals of Botany **19**:281–295. 1905.

[24] MACCHIATI, L., Altri fatti e nuovi argomenti sull' assimilazione fotosintetica fuori dell' organismo dopo le richerche del dig. Dr. Ch. Bernard. Nuovo Giorn. Bot. Ital. **12**:461–468. 1905.

[25] BOT. GAZETTE **41**:157. 1906.

NEWS.

DR. JULIUS WIESNER, the well-known plant physiologist of the University of Vienna, has been made a life member of the upper house of the Austrian parliament.

Dr. BRADLEY M. DAVIS has been spending the spring in Cambridge completing a textbook of botany in co-authorship with Mr. JOSEPH Y. BERGEN. His connection with the University of Chicago will end July 1. He will be at Woods Hole through the summer.

IN VIEW of the great service rendered by Dr. J. BRIQUET at the Vienna Congress, an international demonstration in his honor was arranged. The funds obtained from fourteen different countries have been used in the purchase of a gold watch with congratulatory inscription, a check for 2000 francs for the furtherance of Dr. BRIQUET'S scientific work, and an illuminated address. The details of this movement have been published and also Dr. BRIQUET'S letter of thanks.

THE THIRD annual meeting of the Botanical Symposium will be held from July 2 to 9, 1906, at Mountain Lodge, Little Moose Lake, Old Forge, N. Y. Through the courtesy of the members of the Adirondack League Club the privilege of occupying the Club House for one week is extended to the members of the Symposium. Botanists are requested to notify Mr. Joseph Crawford, Secretary, 2824 Frankford Avenue, Philadelphia, Pa., if they intend to attend the Symposium.

A NEW JOURNAL bearing the title *Annales de Biologie Lacustre* is to be published under the editorship of Dr. ERNEST ROUSSEAU, with the collaboration of a very large board of editors. The first fascicle as announced contains 192 pages with figures and maps. Publication is to be in German, English, French, and Italian. Each volume will contain 400 to 500 pages, and the subscription price will be 20 to 30 francs. The address of the editor is Musée royal d'Histoire Naturelle, rue Vautier, 31, à Bruxelles.

A MOVEMENT is on foot to erect in Jena a statue as a memorial to Professor ERNST ABBE, who died last year. The American Microscopical Society has issued a circular letter appealing to its members to aid in this movement. The Bausch & Lomb Optical Company of Rochester, N. Y., which has long had business relations with the Zeiss works, has also sent out letters asking for contributions from those who are not members of the society. Contributions in any amount will be welcomed and will be acknowledged.

A FASCICLE OF KEW BULLETINS has appeared recently, bearing various dates from 1900 to 1906. This is an attempt to revive a dormant publication suffi-

ciently to permit the annual volumes to be bound. Heretofore these volumes have been represented by the annual appendices, which led to the current gibe that the *Bulletin* had succumbed to appendicitis. The most curious illustration of "closing up ranks" is the volume for 1900, the body of which consists of 32 pages, now issued as nos. 157–168, and which were necessary as a preface to the four appendices.

PROFESSOR W. A. KELLERMAN recently returned from his second collecting trip to Guatemala. On account of quarantine regulations (because of yellow fever) he was obliged to return three weeks before the time set. The party traversed the entire country from east to west and went up as far as Quetzaltenango (alt. 2500^{m}). Collections were made about Lake Amatitlan and also at the still more beautiful Lake Atitlan, and on the ascent of three volcanoes. Perhaps ten times as many species of parasitic fungi were gathered as in the same time last year, and the collections seem to contain many new species.

DR. F. CAVARA reports as reasonably successful the attempts to establish an alpine garden on the slopes of Mt. Etna. It is located behind the Casa Cantoniera at an altitude of 1880^{m}, the first cultures at 1440^{m} having failed on account of the heat and drought. About 150 species are now thoroughly established, and nearly 400 more are more or less sucessfully grown. The garden is surrounded by a stone wall which mitigates the violence of the winds. Cisterns and snow magazines (there are no streams) eke out the scanty supply of rain in the growing season, which in 1904 was 56^{mm} in May, June, July, and August. The director is to be congratulated on overcoming the many difficulties and solving so many of the problems which confront him in this undertaking. The garden has been christened *Gussonea*, in honor of "un valoroso studioso della flora sicula."

GENERAL INDEX.

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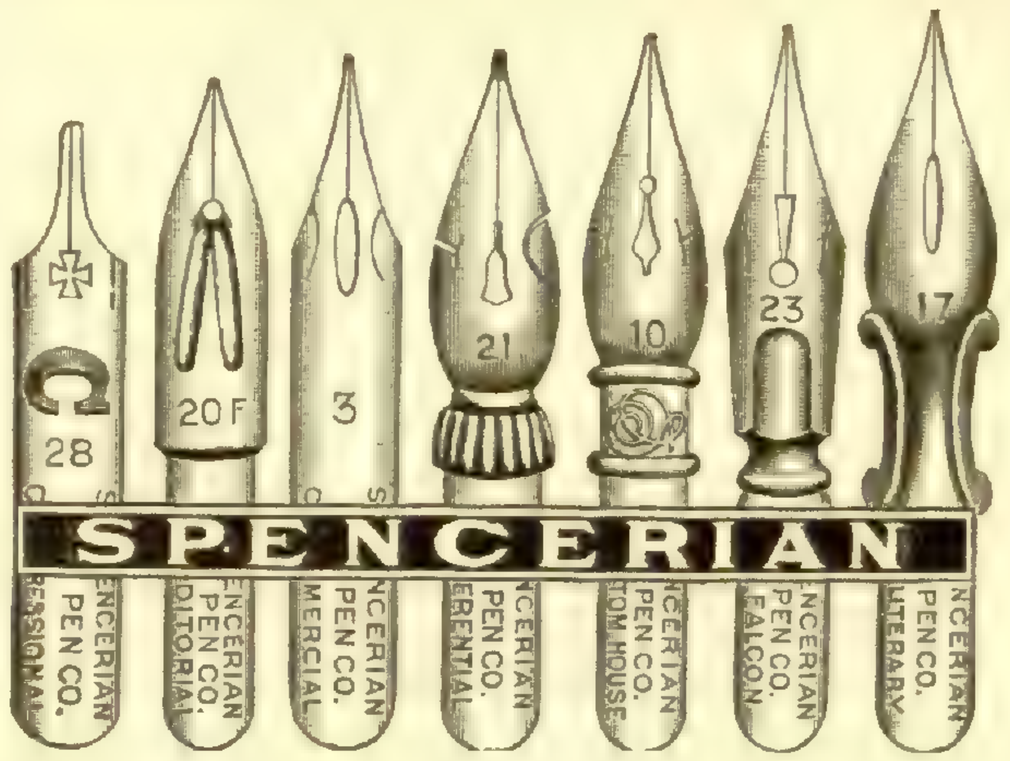

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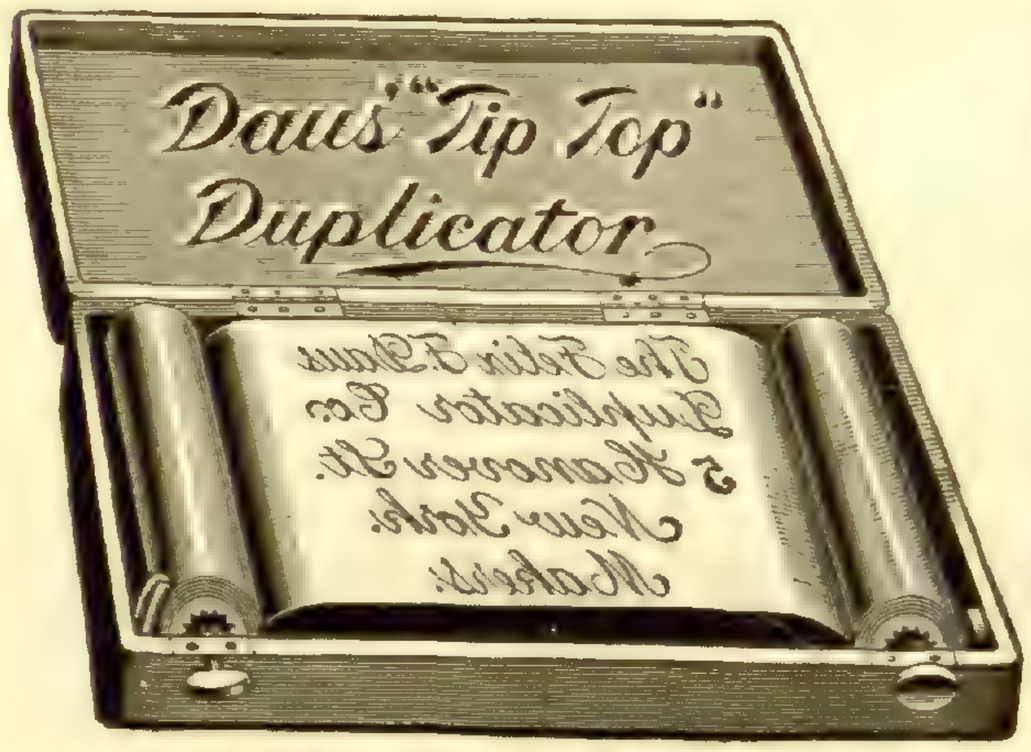

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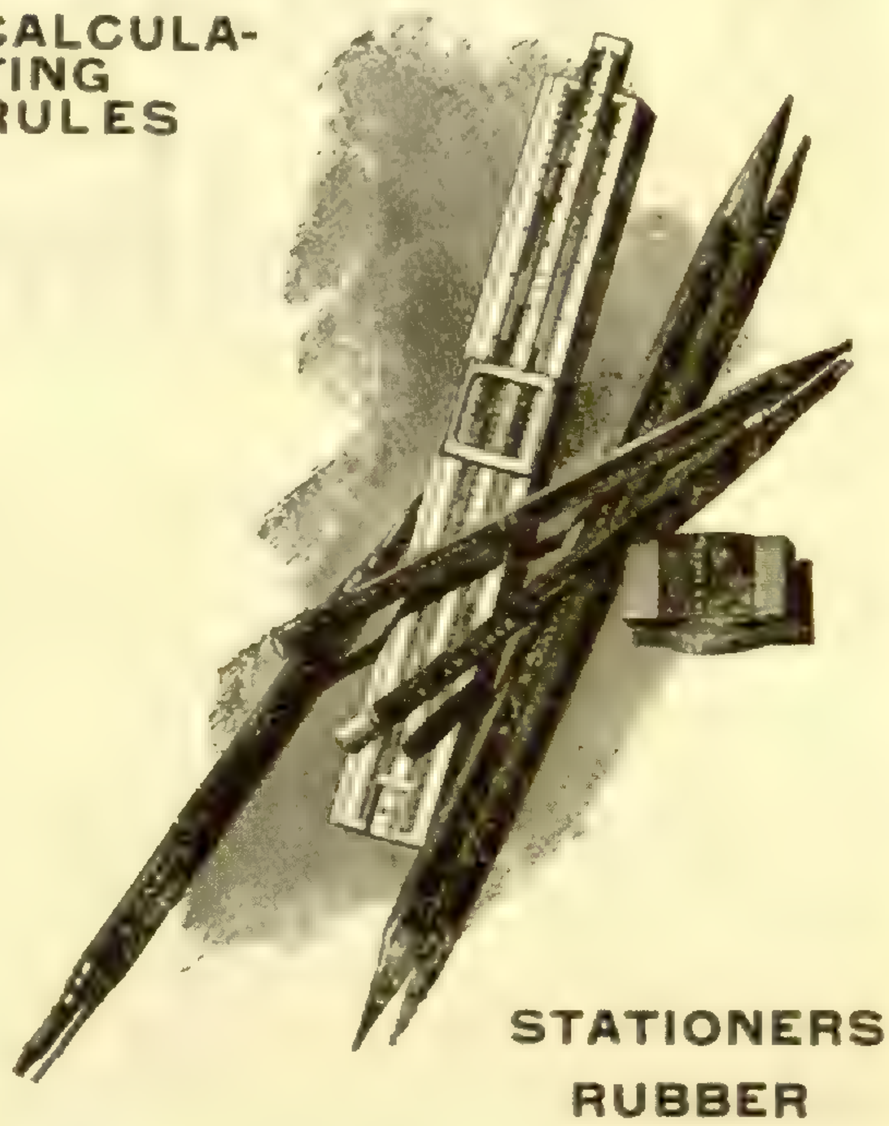

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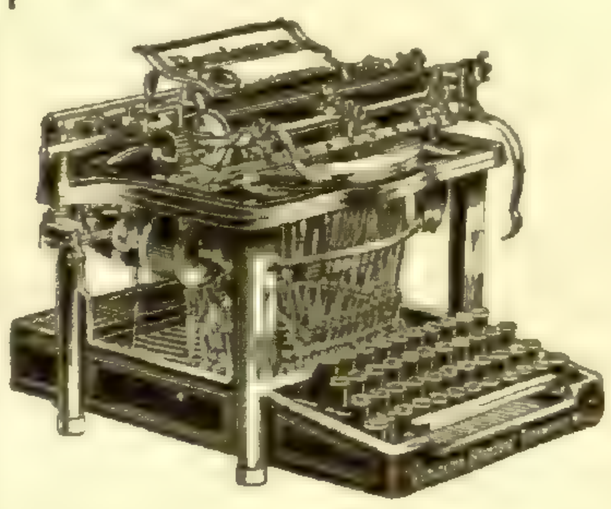

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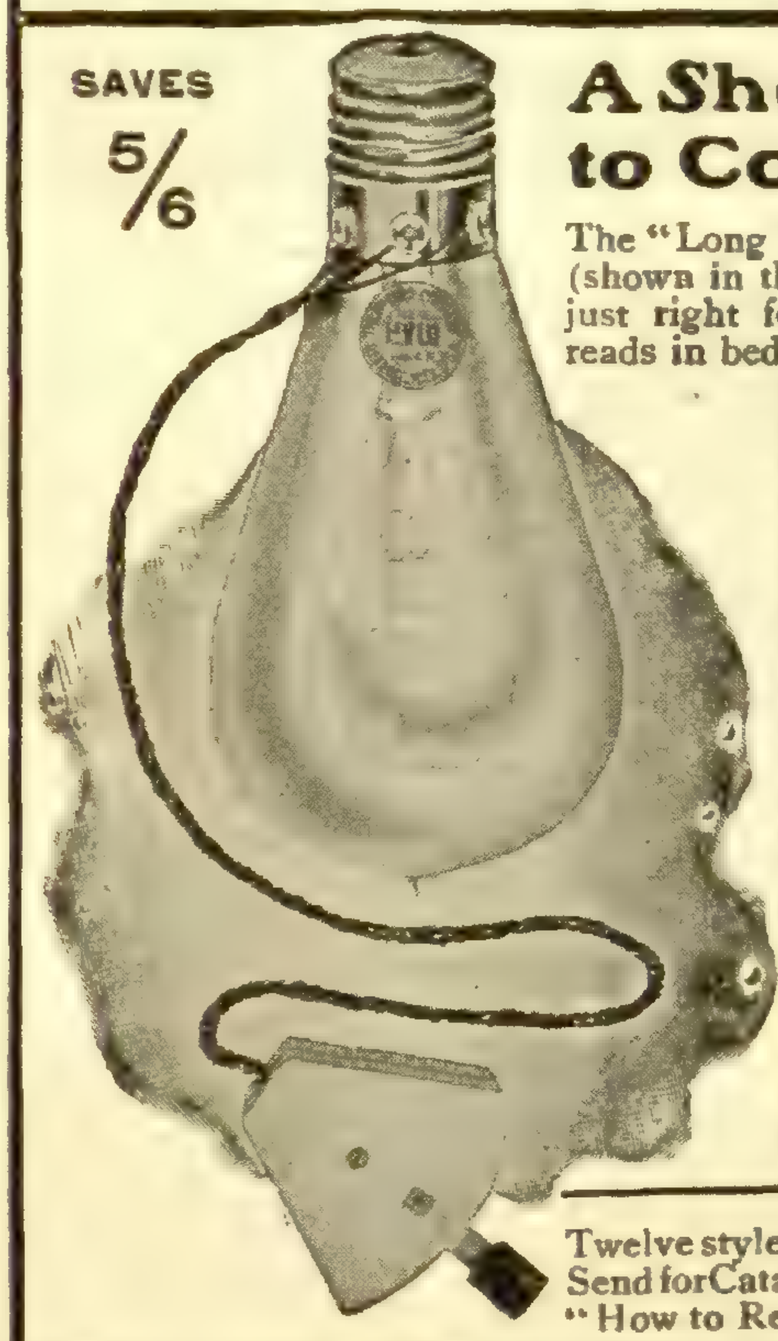

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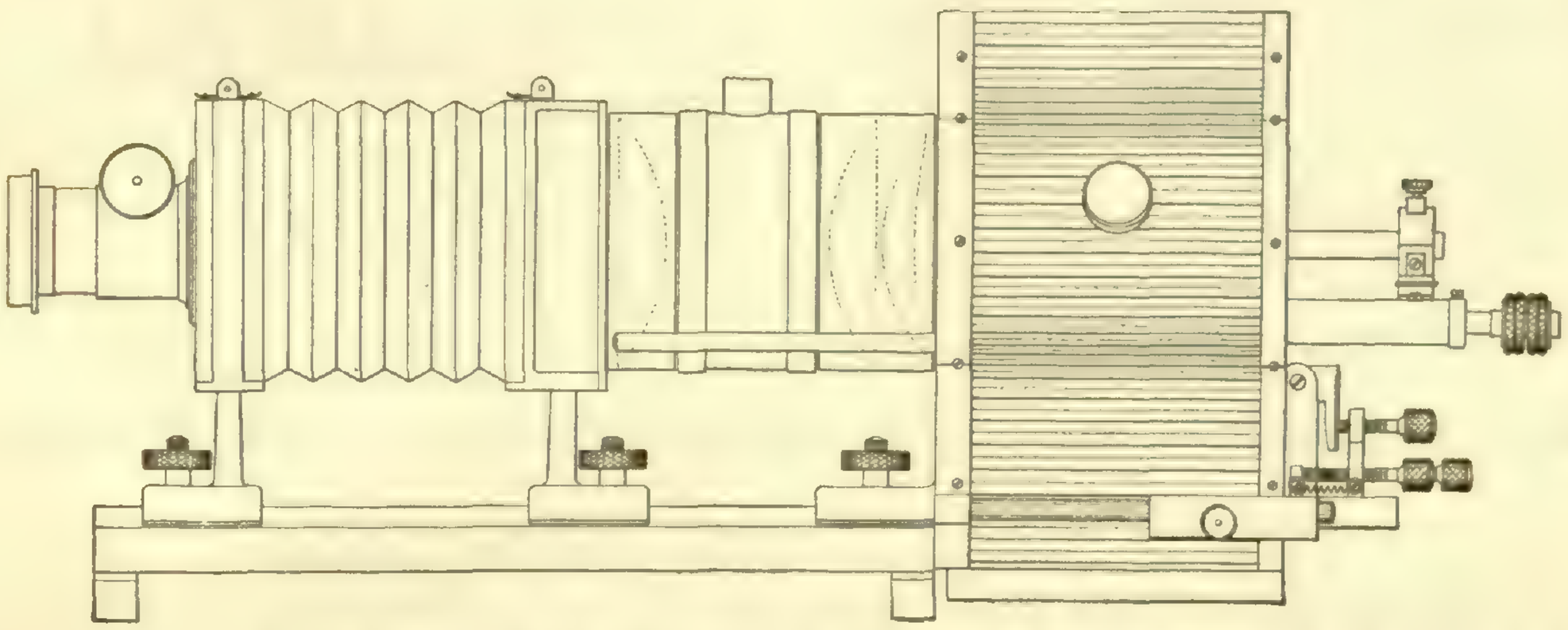

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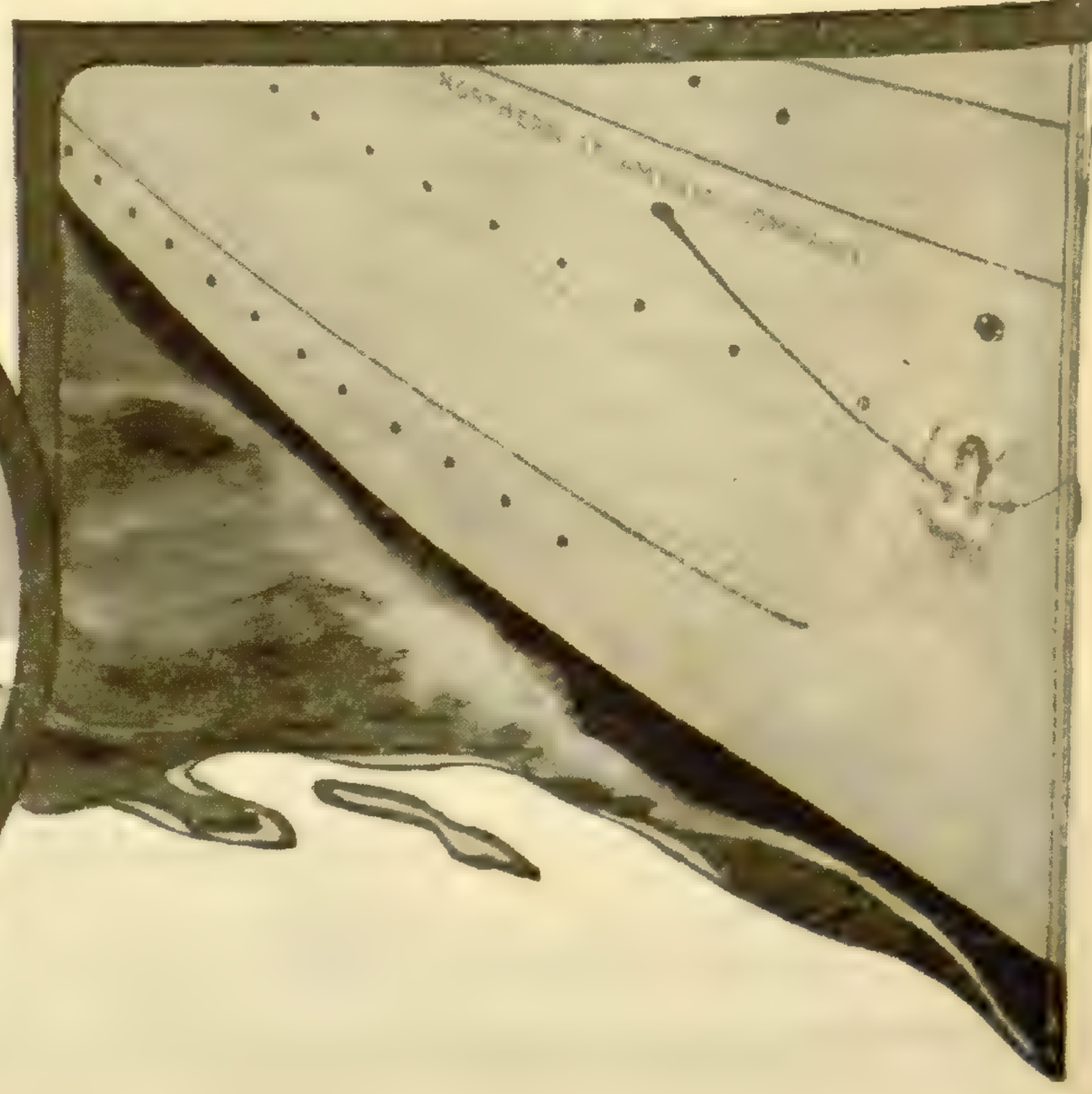

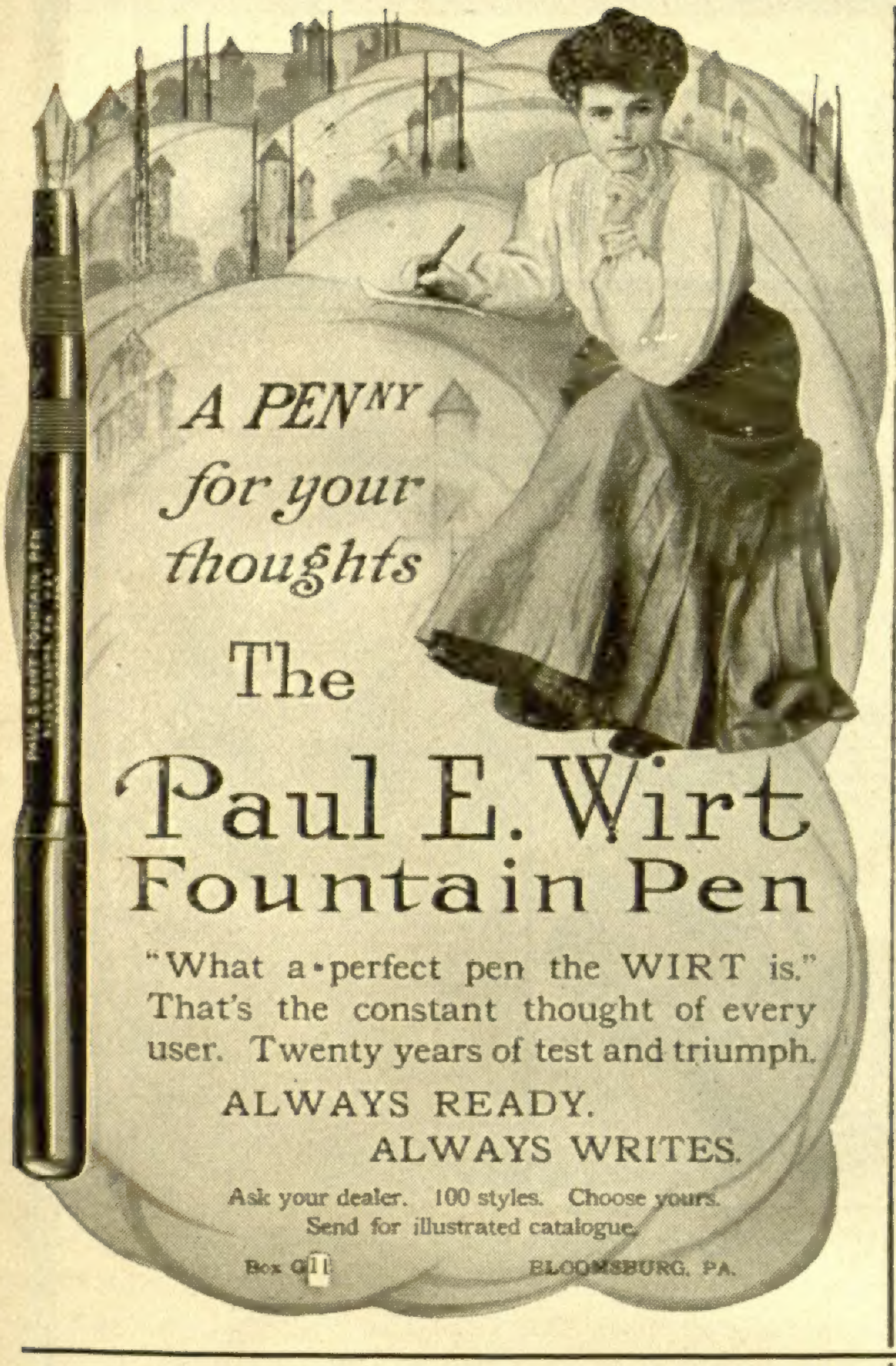

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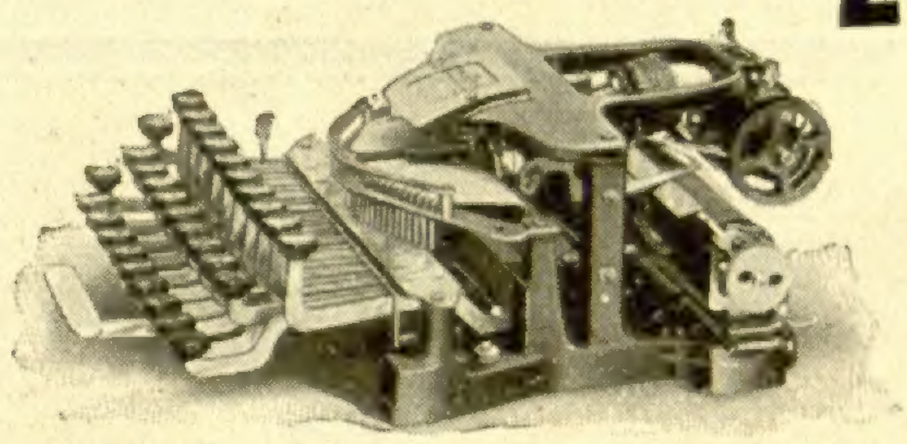

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